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THE FUNCTIONAL REACTIONS OF THE HUMAN THYROID *

A CONTRIBUTION TO ITS HISTOPHYSIOLOGY

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Many attempts have been made to get a better understanding of the histophysiology of the thyroid gland. If Graves' disease is nothing more than a severe condition of hyperthyroidism the structure of the enlarged gland should give us the key to the problem. Unfortunately the arguments in favor of a condition of dysthyroidism cannot be discarded altogether. On the other hand, the histology of toxic goiters (exophthalmic and non-exophthalmic) is by no means a constant one and the efforts made thus far to explain the severity of the clinical course by the histological features have not met with uniform success. The statistics of Wilson [1] published in 1914 show that the clinician and the pathologist are in agreement in only 75 per cent of the cases examined.

The use of the tinctorial reactions of the colloid, suggested by Kraus [2] and Troell,[3] has not improved this percentage by any great degree. However, the concept of the "Wucherungspolstern" introduced by Sanderson-Damberg [4] has enabled Hellwig [5] to get more satisfactory results. This tends to prove that a better knowledge of the finer histological structures will clear up many questions of interest in the toxic goiter problem. Unfortunately, researches along these lines are now hampered with new difficulties which arise from the prevailing Plummer preoperative treatment with iodine (Mayer and Fürstenheim [6]). The accumulation of colloid is a disturbing factor in the interpretation of the morphological findings.

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Another method of approach would consist in looking for distinctive morphological peculiarities of endemic goiters with or without hyperthyroidism. Here again a uniform morphological standard is lacking. Nothing enables us to say whether an enlarged gland does or does not elaborate more thyroxin. As Wegelin [7] points out, our shortcomings may be due to several causes, among which our imperfect knowledge of the histophysiology of the thyroid is a prominent one.

Progress in this direction is needed and the conditions are too intricate to make headway by the study of goiter material. In our opinion progress will be attained only by a scheme of investigation wherein the cellular reactions are closely correlated with a definite functional phase of development, such as metamorphosis (Uhlenhuth [8]), or with a stimulation of the gland measured by the increase of the basal metabolism. The recent work of Okkels, Krogh and Lindberg,[9] who make use of the thyrotropic hormone as a stimulant, opens no doubt a promising field of investigation. Technical difficulties, however, are met with which can be mastered only by a team of workers trained for this particular purpose.

We want to prove in this paper that, in the meantime, useful conclusions can be drawn from a systematic histological and cytological survey of an extensive series of human thyroids collected in the postmortem room. Such material must be fresh, perfectly fixed and should be collected in an area such as Ghent, Belgium, where endemic goiter is very rare.

Investigations of this kind have been carried out in recent years by Farrant [10] and Williamson and Pearse,[11] but their aim was different from ours. For the present at least we are not concerned with knowing whether, in one particular disease, the gland is hyperplastic or not, or whether the gland has a larger or a smaller hormonic output. We use marked changes brought about by pathological conditions to get new information about the functional interpretation of certain histological structures.

It is well known that infectious or toxic conditions sometimes considerably alter the thyroid morphology. What is more often overlooked is the fact that structures appear which are considered by many as a characteristic feature of toxic goiter. The extensive material at our disposal gives us the opportunity to link up a complete series of transitional changes, the study of which leads

to a satisfactory explanation of the extreme stages. We are thus able to connect the morphology with certain aspects of functional activity.

The Morphology of the Thyroid Epithelium during Follicular Depletion (Colloid Release)

The first point we shall consider is the morphology of the thyroid epithelium during follicular depletion. The work of our predecessors, as well as our own investigations, shows that colloid release is a common occurrence in toxic or infectious processes, but it is by no means constant. In 6 cases of pneumococcus lobar pneumonia we never saw any sign of it. Neither did we notice any depletion in acute streptococcal puerperal septicemia. In acute peritonitis following appendicitis, on the other hand, we found regularly a certain degree of colloid release. In patients dying from intestinal obstruction (11 cases) all the gradations of the process exist, so that finally the follicles are found to be reduced to canalicular formations which resemble in all respects similar structures seen in toxic goiters. The same remark applies to diphtheria cases, although in these there is no general rule, intercurrent infection probably accentuating the depletion.

Figures 1 and 2 represent the thyroid of a female, 54 years old, who died within 48 hours after the onset of intestinal obstruction; Figure 2 particularly shows the early stages of the depletion. Many follicles, however, are still filled to a maximum, as in Figure 1. The morphology of the epithelium offers many striking features. First of all, the epithelial lining is heterogeneous, contrary to what is generally stated in standard textbooks. To our knowledge only Aschoff [12] has laid stress on this fact. A narrow segment of high cylindrical cells with dark hyperchromatic nuclei is very evident, while the remaining epithelium is either low cuboidal or endothelioid. Thus we already can distinguish three types of epithelium in one follicle, low cuboidal (Type 1), columnar (Type 2), endothelioid (Type 4). Let us now examine the follicle on the left of Figure 2 and neglect for a moment the typical "Sanderson Polster." The high cylindrical segment is slightly extended. It is doubtful if in this follicle colloid release has begun. We should like to draw attention to the appearance of another type of cell, especially in the

groove adjacent to the "Polster." It is a high cuboidal cell with a large vesicular nucleus (Type 3). A general survey of 500 human thyroids from normal and pathological cases proves that all the varied aspects of the thyroid epithelium can be classified under these four types.

The follicle on the right of Figure 2 shows an unquestionable depletion. Its shape tends to be more or less triangular, while the high cylindrical epithelium extends over two-thirds of the contour.

In Figure 3 again we notice the close connection between the extension of this type of epithelium and the progressive collapse of the follicle. On the left the shape of the two adjoining follicles A and B proves that they are completely filled with colloid. A high cylindrical segment is easily recognizable in both, but it is narrow. On the right of this figure (follicles C and D) these high cylindrical segments extend over the greater part of the follicular wall, while the colloid depletion is already marked. This is still more convincingly emphasized in Figure 4 from another case of intestinal obstruction. Here the follicle is completely surrounded by high palisade epithelium of which the morphology is clearly demonstrated on higher magnification. We need not dwell upon its histological characteristics, except to point out the fact that the width of the cell does not far exceed the width of the oval, hyperchromatic nucleus.

We suggest that there is a close connection between the increase in the size of the segments of cylindrical epithelium and colloid depletion. Since we believe that this point is of importance in thyroid histology, we should like to present further data in favor of this assumption. Figures 5, 7 and 8 represent thyroids from diphtheria cases. Of the four large follicles present in Figure 5 the third from the left shows a beginning stage of depletion. Here the high cylindrical epithelium forms the least extensive segment. In the second and fourth follicles from the left, collapse of the follicle is much more marked. In the one where it is most evident (the fourth), the greatest part of the lining is columnar. Figures 7 and 8 again show this striking correlation; the generalized high cylindrical character of the follicular epithelium corresponds to extreme degrees of colloid absorption. Stress is laid upon the fact that in the human thyroid a large spherical follicle is never found with an entirely uniform, high columnar lining. When this type of epithelium surrounds the follicle entirely, the latter has always attained an extreme degree of de-

pletion. Of course, we find in our preparations sections of collapsed follicles without columnar epithelium. Serial sections prove, however, that they correspond to the tail end of a diverticulum of a depleted follicle, the main body of which, not seen in this particular section, is provided with large high cylindrical segments. Others are depleted follicles of which the epithelium shows regressive changes; they are devoid of functional activity. We may summarize our conception as follows: in a follicle provided with uniform and generalized high cylindrical epithelium, the colloid resorption is always considerable. As shown above the reverse is not true, *i.e.*, a depleted follicle may be found occasionally without cylindrical lining.

The above observations force upon us the conclusion that the columnar epithelium absorbs the colloid stored in the follicle and excretes the active hormone into the blood or lymph vessels. We see some cytological evidence of this excretion in the form of colorless basal vacuoles found only in this type of epithelium, as one of us [13] has shown in a recent paper. They attain a considerable size in some cases of diphtheria, as shown in Figure 7.

Moreover, there is always a very marked vasodilatation of the sinusoids adjoining this type of epithelium. The lymph vessels are also distended; in cases of acute depletion this may lead to edema of the stroma (Fig. 7). The excretory function of high columnar epithelium is in itself not surprising since toxic goiters are so amply provided with it. A study of the latter would have led to this conclusion were it not for some exceptions which resulted in confusion. In the light of what follows, these discrepancies, and especially the eventual absence of high epithelium in a toxic goiter, can be easily explained.

The Effect of Colloid Depletion

The result of colloid release on the architecture of the follicle is illustrated by Figure 7. The collapse of the wall leads to the formation of diverticulums which later on sever all connection with the main follicle. In this way accessory minute follicles are formed which, in some instances, as in Figure 8, surround the depleted follicle as satellites. These diverticulums of the main follicle have been noticed since Virchow by many authors (Wegelin,[7] Marine,[14] Wilson,[15] Norris,[16] and Rienhoff[17]). Most of these workers never took into account the part the disease played in the extension of these

secondary acini and considered the diverticulums or secondary acini as evidence of proliferation. A recent work of Moritz [18] also favors this view. Figure 7 proves clearly that this is not the case. Glands presenting this morphology have a low weight. There is actually no budding but a formation of diverticulums through mechanical factors. However, the collapse of the follicular wall is not the only responsible factor. A modification of some of the constituents of the excretory segment also plays a rôle. In protracted cases where the thyroid has been stimulated for some time the columnar and rather dark cells gradually increase in size while the nucleus becomes large and vesicular (Type 3). The turgescence of a row of adjoining cells forces them to bulge out. The effect of the two combined factors is illustrated most clearly in Figure 6, where we notice not only depletion of the main follicle but also a bulging out of the newly formed diverticulum.

It is evident that, when this process of diverticulum formation extends along the entire follicular wall, as can be seen in Figures 4 and 8 (severe collapse), the process leads to a fragmentation of the main follicle. The key to the interpretation of senile involution of the thyroid lies in this important observation.

The Morphology of Intrafollicular Colloid Secretion (Storing Process)

The colloid represents a storage product which in adults is the result of a very slow secreting process operating from birth. On the other hand, there is ample evidence that after acute colloid depletion the colloid can be restored quickly to normal.

One of us [13] has carefully followed this slow accumulation of colloid in young children who died accidentally. It is a striking fact that up to the age of 12 years the high cylindrical segments are very scanty, so that the morphology of the thyroid epithelium (at this age) is almost uniform and of the low cuboidal type. As this corresponds to a gradual increase in size of the follicles brought about by a process of coalescence we concluded that low cuboidal epithelium slowly secretes colloid into the follicular cavity.

Figure 9 illustrates the appearance of the epithelium during an active process of intrafollicular secretion. The follicle represented in the center of Figure 9 has reached an extreme degree of depletion.

It belongs to a thyroid from a case of intestinal obstruction where the patient survived 4 days. A very narrow segment of high cylindrical cells is still evident. In our opinion these cells represent remaining segments of the excretory (or absorptive) function. The other cells have undergone marked changes. These cells have broadened out, their nuclei have become vesicular and increased in size and they have the characteristics of cell Type 3. They are secreting into the follicular cavity large droplets (Anderson vacuoles) and at the same time a watery, transparent fluid. We see here a striking example of the beginning of a new and very active colloid secretion. A more advanced stage is seen in Figure 10. It is from the thyroid of a child who had passed the stormy period of diphtheria and was actually convalescent. The child died suddenly of heart failure 18 days after the onset of the disease. Small doses of iodine were administered daily during the illness. The thyroid shows marked changes. The follicles are of medium size (80 to 100 microns in diameter) and much smaller than in children of the same age who die accidentally (100 to 150 microns). Most probably this thyroid underwent, during the acute stage of the disease, a period of depletion comparable to that represented in Figures 7 and 8. However, at the time of death the gland was rapidly restoring its colloid material, since almost all follicles are spherical and filled with a thin, transparent fluid. Moreover, evidence of follicle coalescence, such as Moritz [18] recently described in man and Uhlenhuth [8] in the salamander, is frequent. The newly accumulated colloid stands out in contrast to the older, denser colloid, lying in the center of the follicular acinus. Here again, as in the previous preparation (Fig. 9) the epithelium is composed of large cells with large vesicular nuclei (Type 3). If it were possible to present an unlimited number of photographs, we could easily demonstrate the frequency of similar aspects in subacute septic processes, such as peritonitis following appendicitis (7 to 9 days duration).

These observations lead us to the conclusion that the high cuboidal cells (under certain conditions large cylindrical), which contain a large hyperchromatic nucleus and clear cytoplasm with an extensive surface contact with the blood vessels, secrete the colloid into the follicle actively and rapidly. Experimental data support this view. Injection of pilocarpin into guinea pigs and rats increases the intrafollicular mass of colloid and transforms a low cuboidal

epithelium into a high cuboidal type. We also find the latter form of epithelium in rats which, having been exposed to cold, restore their colloid at room temperature. It is well known from the work of Cramer [19] that exposure to cold causes a severe colloid release and it may be interesting to note incidentally that during the depletion period high cylindrical epithelium occurs, a fact that agrees with our conclusions.

The functional significance of endothelioid epithelium is obvious, namely that of a slow secretion of colloid.

The Morphology of the Normal Thyroid

Keeping in mind the functional significance of these different types of epithelium, a survey of human thyroids of adults who died soon after accidental injuries leads to a new conception of thyroid histophysiology. The epithelium of normal control glands is in fact heterogeneous. Although the predominant type is low cuboidal, narrow segments of high columnar cells are present in some of the large follicles. If our previous observations are correct, the number and size of these columnar cell segments should give us an indication of the hormonal output of the gland. They represent the only part of the parenchyma that sets free the hormone into the circulation.

This active part is very small if we consider the fact that only one of five large follicles shows these narrow excretory segments. This observation is in harmony with the histophysiology of other endocrine glands, such as the suprarenal cortex, where the available evidence points to the fact that likewise only a very small proportion of cells is actively at work under normal conditions, while the remainder of the parenchyma is held in readiness for special emergencies.[20]

We have come to the conclusion that in normal glands the high columnar segments can be divided into two groups. An example of the first group may be seen in Figure 1, where there are no accessory follicles. In most instances, however, we notice that they are in close contact with small secondary satellite follicles generally provided with low cuboidal epithelium and formed by a process described above (second group). These peculiarities are convincingly demonstrated in Figure 11, which shows a thyroid from a man 21 years old who was killed in a motor accident.

The "Sanderson Polsters"

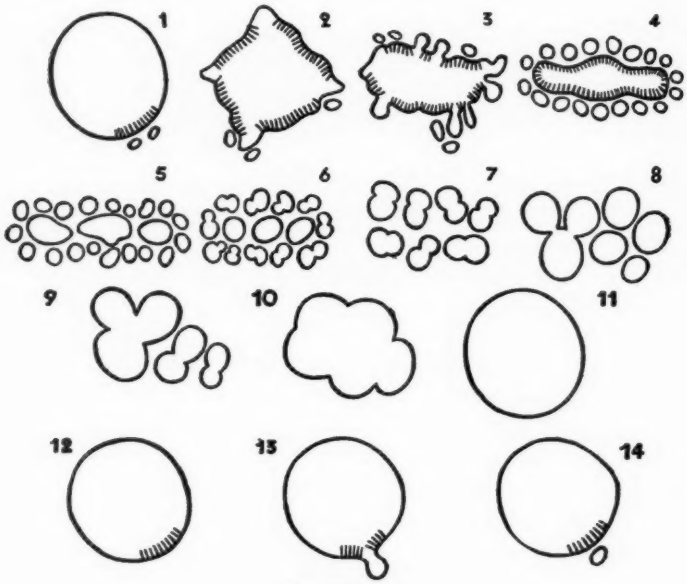
When colloid secretion increases in the small secondary follicles their mass bulges into the follicular cavity and gives rise to a "Sanderson Polster" (Fig. 2) for which we suggest the term "papilla." These secondary acini are nearly always lined with large epithelial cells (Type 3) and the nuclei are conspicuous by their size. These papillae, or "Sanderson Polsters," are a common finding in septic or toxic processes which stimulate the secretion in those secondary acini. Up to the present time the "Sanderson Polster" has been considered a center of proliferation. In our opinion it is the expression of a simultaneous stimulation of hormonal excretion by the columnar epithelium of colloid secretion by the cells with large nuclei, which are restricted to a small portion of the follicle complex. We believe that the papillary formation is due to three factors: increase in size of the cells, increase of colloid secretion and congestion of the capillaries.

These papillae consequently correspond to localized zones of functional activity, normally present, but brought into prominence by the stimulation of physiopathological conditions. In the light of these observations the significance of the small acini is quite different from that of the large follicles. Only the latter excrete the hormone into the circulation. As shown above, the two types are, however, closely related. High columnar cells having performed their excretory function become turgescent and are extruded in the form of acini out of the main follicle, the process being accentuated by the depletion of the latter.

The Functional Unit

We believe, therefore, that the human thyroid is composed of functional units, *i.e.*, complex formations of main and secondary follicles. In many cases of severe sepsis these histological features are evident and no better illustration can be given than by Figure 8. Under normal conditions the functional units are less conspicuous because the colloid storage overshadows the signs of colloid absorption. The greater part of the epithelium continues to increase the storage of colloid slowly, while in some main follicles only a small segment of cells excretes the hormone into the capillaries (Figs. 1 and 11). The concept of the functional units was brought

forward by Williamson and Pearse,[11] but their argument is weakened by the excessive stress they lay upon the significance of the lymphatic system, which in fact does not differ essentially from that of any other endocrine gland. The same concept has been implied by those workers who have made reconstructions of the thyroid follicle since the publication of Wilson.[15] However, the only way to obtain convincing evidence is to make a study of glands profoundly changed by the physiological effect of pathological conditions.



TEXT-FIGURE 1

In health and disease the active functional units are ever-changing structures. With a constant number of cells the functional demands of the gland are met by various appropriate cell combinations.

In the early stages of a septic or toxic process the thyroid unit reacts by a stimulation of both excretion and secretion, which compensate for each other. The segments of high cylindrical cells extend, while the low cuboidal cells of the main follicle change into cells with large nuclei (Type 3), a process which brings the "Sanderson Polster" into prominence. This period of *compensated activity*

lasts a variable time. In 5 cases of streptococcic puerperal infection we found evidence of it from 8 to 10 days after the onset of fever. In peritonitis following appendicitis, in several cases of diphtheria and in protracted cases of staphylococcic pyemia, there is evidence of an early depletion. The *decompensation* is still more pronounced in intestinal obstruction. On the other hand, several cases of peritonitis following appendicitis which we have studied prove that the depletion and fragmentation can be followed by a restoration period leading, through a process of coalescence of the follicles, to the normal configuration of the functional unit (Text-Fig. 1).

The succession of periods of colloid release and colloid secretion appears as a fact of general significance in the histophysiology of the thyroid. Uhlenhuth's [8] remarkable work proves that metamorphosis in *Ambystoma opacum* requires an activity of the thyroid which leads to a considerable colloid depletion. However, a period of active restoration of the colloid soon follows. Kuhn,[21] on the other hand, noticed this same succession of hormonal excretion and intrafollicular colloid secretion in salamander larvae injected with thyrotropic hormone of the anterior lobe of the pituitary gland. In man (Van Goor,[22] Schmelling [23]) and in mammals (Benazzi [24]) colloid release is observed in the later part of fetal life. As Benazzi has shown, the follicles at birth are already refilled when the animal is born active (such as the guinea pig or the lamb). In other mammals, such as the mouse and rat (Benazzi) and man (own observations) the colloid restoration takes place soon after birth.

Discussion

We are well aware that our conclusions may lead to controversy. Any one familiar with the histology of toxic goiter will be prepared to accept the excretory function of the high columnar epithelium (*i.e.*, colloid absorption or release with hormonal output into the circulation). In fact, Holst[25] has already suggested that the absorption process takes place at the site of the "Sanderson Polsters." But this idea was more or less hypothetical. The critical mind will point to toxic goiters devoid of this type of epithelium. If Graves' disease or thyrotoxicosis centers entirely in the thyroid, which is far from being an established fact, our morphological studies suggest that, besides the unquestionable multiplication of its cell constituents, the thyroid shows the decompensated type of hyperfunction when no pre-

operative iodine treatment has been used. The functional units resemble in many respects those of a case of diphtheria, apart from the hyperplastic characteristics (Fig. 8). We have shown that in this type of hyperfunction a phase of colloid restoration very often follows the phase of depletion. In appendicitis there is even evidence that both processes alternate. The discrepancy in the histological findings in goiter material may be easily explained by the fact that the gland is removed during such a period of restoration. This temporary lack of thyroxin excretion need not necessarily correspond to an improvement of the symptoms, since the thyroxin is very slowly destroyed in the tissues.

There are other objections to be met. One could argue that the colloid depletion is not the result of the functional activity of columnar cells but that the morphology of the thyroid epithelium is controlled by the collapse of the follicle. This argument does not hold. In rats exposed to cold the small size of the follicles, or more exactly the difference between their diameter and the height of the cells, is so slight that collapse is not possible and yet high cylindrical cells appear at a given moment during the experiment.

In the literature columnar epithelium has generally been interpreted as a sign of hypertrophy and has been associated with cell proliferation. Marine [14] says that "columnar epithelium always indicates hypertrophy." With this we cannot agree. Columnar epithelium is a normal constituent of the gland. Its scarcity explains why it has been overlooked up to the present. In any human control gland we have had no difficulty in finding columnar epithelium grouped in narrow rows. When in simple colloid goiter these columnar cells become very evident, Wegelin,[7] Aschoff [12] and Hellwig [5] consider it a sign of proliferation. There is no convincing argument in favor of this assertion. In our opinion the increase in number and in size of these columnar segments coincides with the onset of toxic symptoms, and indicates that the hormonal output is increased. This is much more in agreement with clinical observations. It will be interesting to reëxamine simple colloid goiters from the point of view of the existence of columnar segments. If we find them regularly a much debated problem will be solved, we shall understand at last why in spite of their low cells most of these goiters are not accompanied by thyroid insufficiency. Aschoff,[12] who has based his classification partly on the existence of

the so-called proliferating buds provided with columnar epithelium, admits that the presence of the latter coincides with the appearance of symptoms of hyperthyroidism. If we compare these observations with ours, it must be conceded that they lend support to our views.

The chief argument of those who believe that high cylindrical epithelium indicates cell proliferation consists in the fact that adjacent to this type of epithelium there is always an active process of budding. That is why the Sanderson formations have been considered proliferation zones. To this we answer first of all, that in no freshly fixed human gland does one find isolated cells budding from high epithelial cells; they are always grouped in acini with a definite lumen. Secondly, we point again to the mode of formation of these secondary acini where only mechanical factors play a part.

We do not deny the possibility of a multiplication of cells in the papillae, but so far no one has given decisive proof of it. In our fresh human material we have never found mitotic figures. Of course we do not lay stress on this because we are dealing with agonal conditions. However, in thyroids of rats, guinea pigs and rabbits we have repeatedly found mitotic figures in both low and high epithelium with the same frequency. In exophthalmic goiter, where the different types of cells described above are present, mitoses are found in any one of them. What is still more convincing is the fact that the weight of the glands with a preponderance of columnar epithelium (Figs. 7 and 8) is less than the average (5 gm. instead of 7 gm. average weight; 3.5 gm. instead of 5 gm. average weight). We had the opportunity of examining several specimens of thyroids from males and females between 50 and 65 years of age killed accidentally. Extensive segments of columnar epithelium were present, as well as secondary acini. If these formations indicate proliferation why should the gland decrease in weight, as our measurements have shown? In the thyroid of salamanders Uhlenhuth noticed also that mitoses were scanty at a period when papillae with high cells were numerous.[8]

Moreover, recent observations support the view of the excretory function of columnar epithelium. As mentioned before, Van Goor [22] and Schmelling [23] have proved that in the later part of human fetal life the colloid is released to a certain extent. In fresh material we find that during that period, and especially at birth, the epithelium is chiefly columnar. Moreover, in the experiments of Okkels, Krogh

and Lindberg [9] where the hormonal excretion was measured by the basal metabolism, typical columnar epithelium appeared, as is shown in Figure 3 of their publication.

At the onset of metamorphosis of *Ambystoma opacum* the functional activity of the thyroid is unquestionable. Although Uhlenhuth [8] does not distinguish different cell types it is evident from a perusal of his paper that columnar epithelium is present during the colloid release. We should like to call attention especially to Textfigure 16, page 645 of his paper published in *Arch. f. Entwcklngsmechn. d. Organ*, 1927, **109**. The appearance of columnar epithelium during colloid release is also strikingly demonstrated in a series of contributions by Corti's pupils on the thyroids of birds coincident with the development of feathers.[24, 26]

We shall not discuss at length the secretory function of the cells of Type 3 the surface contact of which with the capillaries is always considerable. In fact it is with those cells as well as with the low cuboidal ones that our predecessors have been chiefly concerned. We refer to the classical work of Bensley and the remarkable contributions of Uhlenhuth.[8] We hesitate to dwell on their cytological features for fear of interrupting the unity of our argument. More about this question will be found in a paper by one of us.[13] We believe that Langendorff cells are compressed cells without any functional significance. Furthermore, we shall not take up the question of the inversion of polarity set forth by Cowdry or the significance of the modifications of the colloid and the Anderson intra-acinar vacuoles (Uhlenhuth,[8] Aron [27]) which are no doubt related to an increased activity of the gland. The Anderson vacuoles pass from the cell into the follicular cavity. Our photographs and Uhlenhuth's drawings entirely agree. The Anderson vacuoles constitute one of the aspects of rapid intrafollicular secretion and in Uhlenhuth's observations, as well as in our own, their number attains a maximum when the colloid restoration is well on the way.

It will be noticed that we have not mentioned the Wölffler cells or solid interstitial cell groups. Nothing in our extensive investigations supports their embryonic nature. In normal childhood there are no isolated interstitial cells or cell groups. They appear only when the functional units have been stimulated (see also Aschoff,[12] and Rienhoff [17]) and broken up into small follicles through protracted toxic or septic conditions, or through senile involution.

They are formed only when small or medium sized follicles are predominant.

We have come to the conclusion that the parafollicular cells (Nonidez [28]) of small mammals are homologous to the small satellite follicles of the human thyroid. Mechanical conditions (small size of the follicle) prevent the collapse and folding of the follicle wall and only the second mechanical factor mentioned previously plays a part, namely the turgescence of the cell. The process has been clearly described by Florentin [29] and Nonidez.[28]

Bernard,[30] Benazzi [24] and Florentin [29] have suggested that these cells or cell groups excrete the hormone actively into the blood stream. Examination of material from small mammals may favor this view, as does also the cytological structure of these cells and their close connection with the blood vessels. However, a wide experience with the human thyroid compels us to discard entirely this opinion. Furthermore, the predominant histological features of toxic goiter are completely opposed to this theory.

Conclusions

1. Four types of epithelium exist in the normal human thyroid: (1) the low cuboidal type which secretes colloid slowly into the follicular cavity; (2) the large high cuboidal or broad cylindrical cell type with large nuclei which secretes colloid actively into the follicular cavity; (3) the columnar type which absorbs the stored colloid and excretes the hormone into the blood or lymph circulation; and finally (4) the endothelioid type which is associated with a very slow colloid secretion.

2. The thyroid cells are grouped in ever changing functional units which under normal conditions differ from each other by their hormonal output.

3. The units containing narrow columnar cell segments (a normal constituent of the gland) excrete the hormone actively into the circulation.

4. These active functional units are composed of a main follicle and satellite follicles. The excreting zone of columnar cells is always located in the main follicle and usually in close connection with satellite follicles. The remaining epithelium of the functional unit is for the most part low cuboidal and under certain conditions

endothelioid. Occasionally some cells with large nuclei are present in the satellite follicles.

5. Under normal conditions the amount of stored colloid is kept fairly constant by the compensatory activity of columnar epithelium on the one hand and cuboidal epithelium on the other.

6. The small satellite follicles are derived from the excretory segment, not as the result of a budding process but through the action of two mechanical factors: (1) the folding of the excretory epithelium following depletion, and (2) the turgescence of the columnar cells that have exhausted their excretory function. The latter factor may operate alone. The small satellite or secondary follicles represent cell groups beginning anew their functional cycle.

7. There are no interstitial cells or solid interstitial cell groups present in the normal infant or adult thyroid, while in senile involution they may become apparent. This observation refutes the embryonic nature of these elements.

8. "Sanderson Polsters" are the result of functional stimulation of the excretory zone which expands, and of the group of underlying acini which secrete colloid more actively. This papillary formation is caused by an increase in size of the cells, an increase of the colloid secretion and vasodilatation.

9. Although we do not deny the possibility of cell multiplication in high columnar segments or in the "Sanderson Polsters" we maintain that they are not specifically proliferation centers under normal conditions.

10. A very small proportion of thyroid cells excretes hormone into the circulation.

11. Under pathological conditions the gland reacts at the beginning by an extension of the columnar excretory segments and by the transformation of the low cuboidal type into the cell type with large nuclei. At this stage there is no depletion. Increased hormonal excretion into the circulation is compensated for by increased intrafollicular secretion. This compensated stage may last 10 days (streptococcic septicemia). In cases of peritonitis following appendicitis, intestinal obstruction, and occasionally in diphtheria, there is evidence of an early decompensation, the excretion predominating over the intrafollicular secretion.

12. The depletion of the main follicle leads to the formation of secondary acini and finally to extensive fragmentation of the func-

tional unit. The latter may be restored to its normal features through the secretory activity of the small follicles which fuse together. There is evidence of alternating periods of colloid release and colloid storage during protracted infectious or septic diseases.

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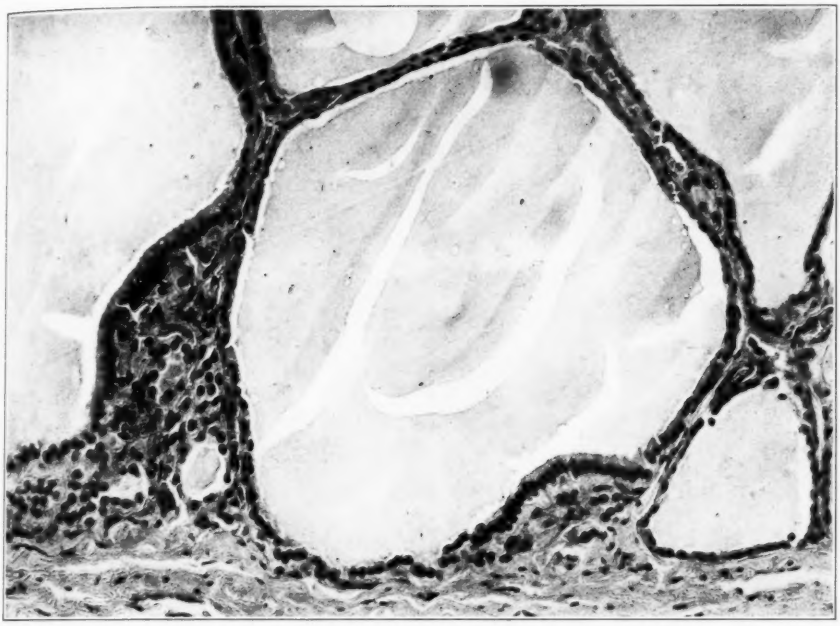
DESCRIPTION OF PLATES

We are indebted to Mr. F. Pittock for the photomicrographs. These were made through the kindness of Professor J. P. Hill, of the Department of Histology, University College, London.

Plate 153

Fig. 1. Thyroid from a woman 54 years old. Intestinal obstruction by strangulated umbilical hernia. Survival 2 days. Distended follicle. Heterogeneous epithelial lining. Narrow columnar segment slightly protruding into the follicular cavity. Turgescence of the nuclei of the underlying sinusoids. × 300.

Fig. 2. Follicle from the same case showing slight colloid release. Four types of epithelium — endothelioid, low cuboidal, cells with large nuclei and columnar. The columnar is larger than in Fig. 1. Vasodilatation of the underlying capillaries, satellite follicles. "Sanderson Polster" protruding into the follicular cavity. × 300.



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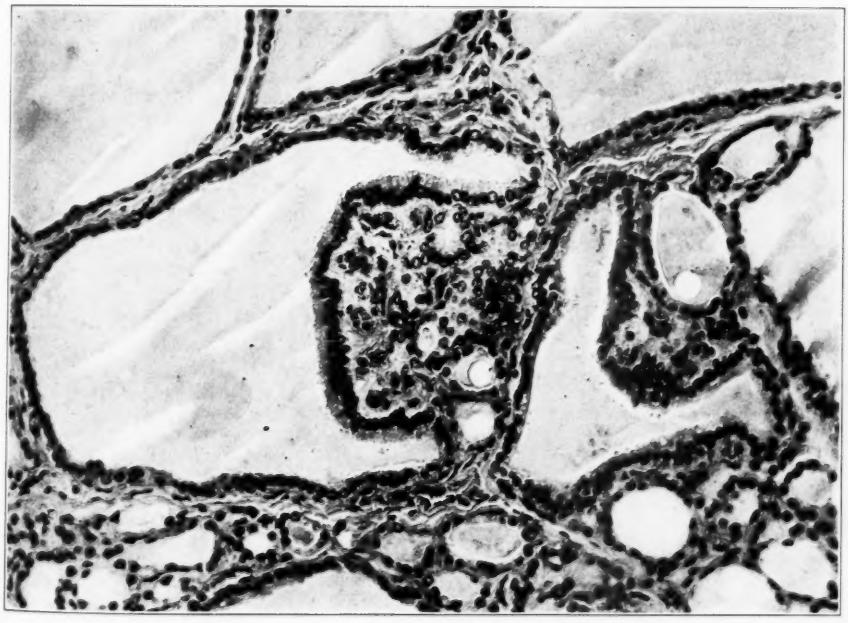

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Plate 154

Fig. 3. Thyroid from the same case at lower magnification showing the correlation between the extension of columnar epithelium and colloid depletion. × 150.

Fig. 4. Thyroid from a woman 70 years old. Intestinal obstruction caused by a scirrhous carcinoma of the sigmoid colon. Survival 10 days. Advanced stage of colloid depletion. The follicle is surrounded by uniform columnar epithelium. Note apical colloid granules. Marked formation of diverticulums and satellite follicles provided with a lining of cells with large nuclei (Type 3). × 500.

3

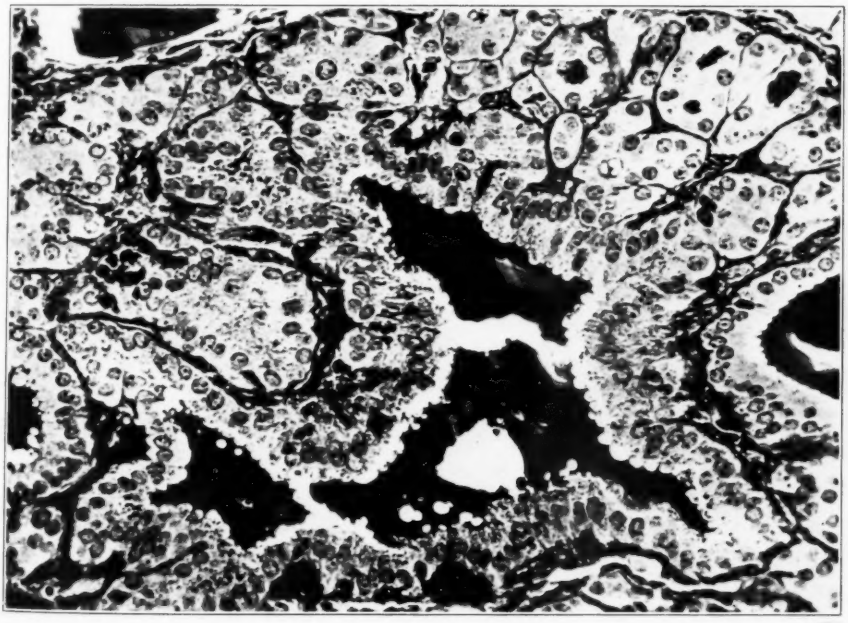

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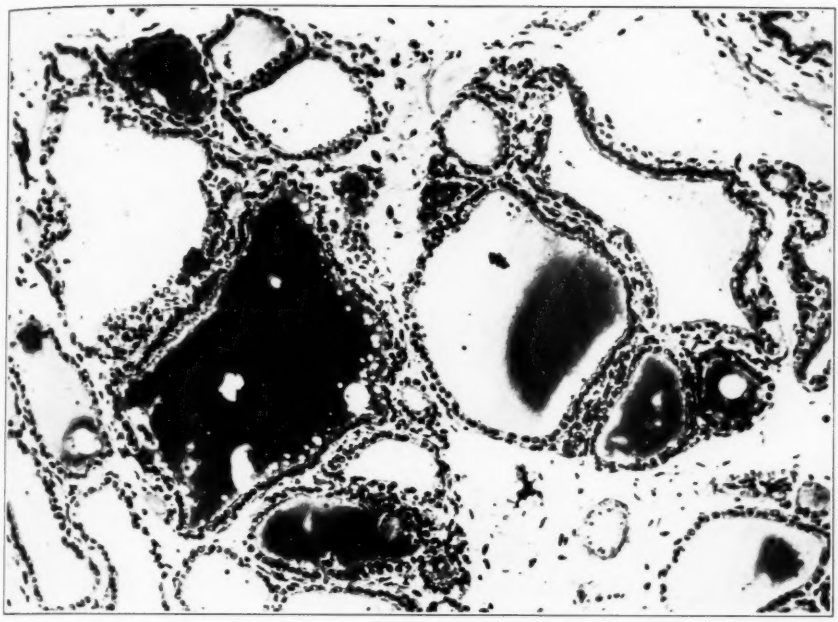
Plate 155

Fig. 5. Thyroid from a girl 7 years old. Diphtheria, toxic symptoms, glomerulonephritis. Region of the thyroid showing the correlation between the extension of columnar epithelium and colloid depletion. × 230.

Fig. 6. Thyroid from a woman 54 years old. Intestinal obstruction. Morphological features of diverticulum formation (cells with large nuclei). × 230.

Fig. 7. Thyroid from a girl 7 years old. Diphtheria. Acute clinical course. Severe depletion of a follicle. Diverticulum formation. Note the clear zone at the bottom of each cell. Edema of the stroma. × 300.



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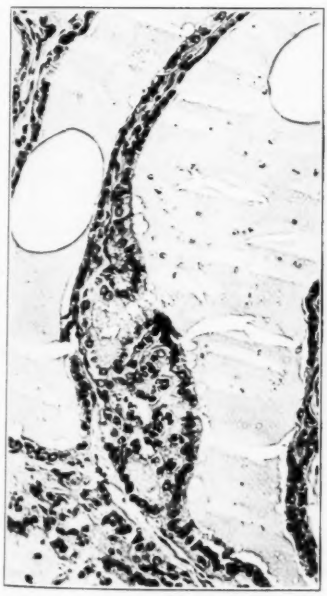

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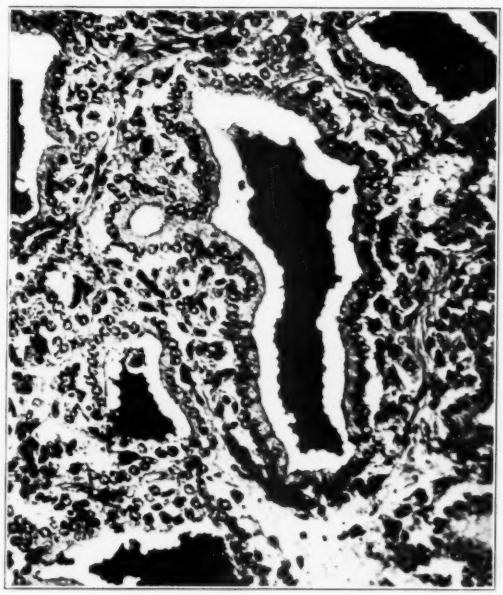

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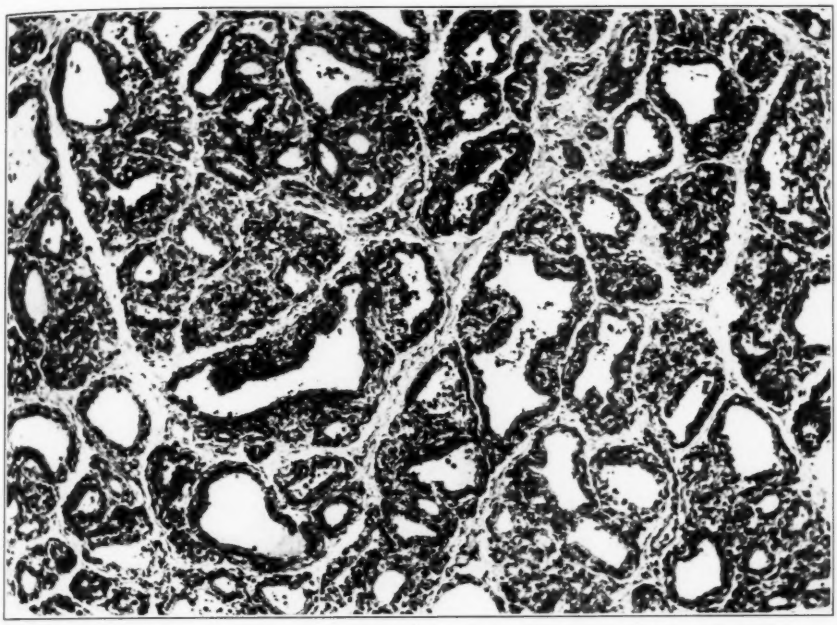
Plate 156

Fig. 8. Thyroid from a girl 5 years old. Acute diphtheria. Severe colloid depletion. Extensive development of columnar epithelium. Marked formation of satellite follicles. Functional units very evident. × 150.

Fig. 9. Thyroid from a woman 57 years old. Intestinal obstruction by strangulated umbilical hernia. Survival 4 days. In the center of the photomicrograph is a collapsed follicle filled with a clear colloid. Note a narrow columnar segment. The remainder of the epithelial lining is composed of cells with large nuclei. Secretion of apical vacuoles (neosecretion). × 500.



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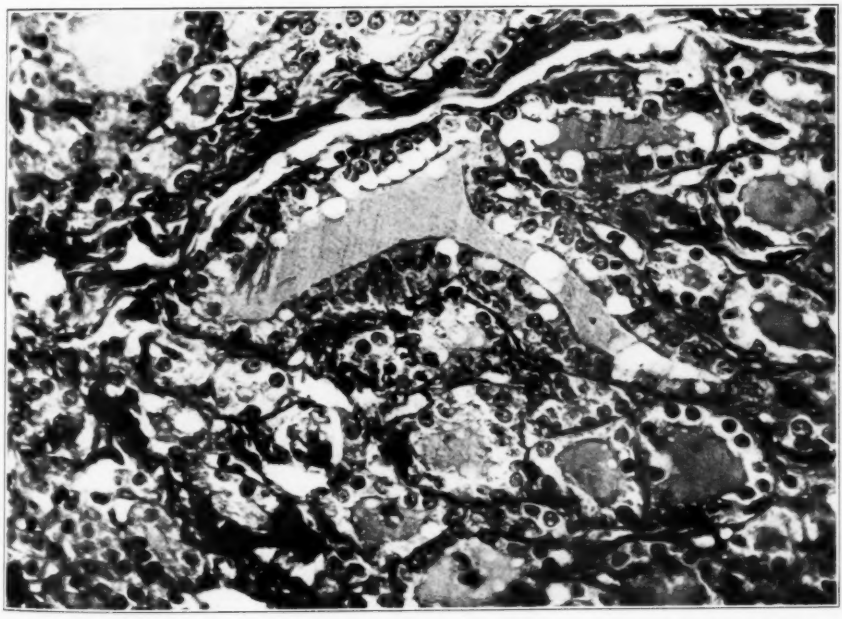

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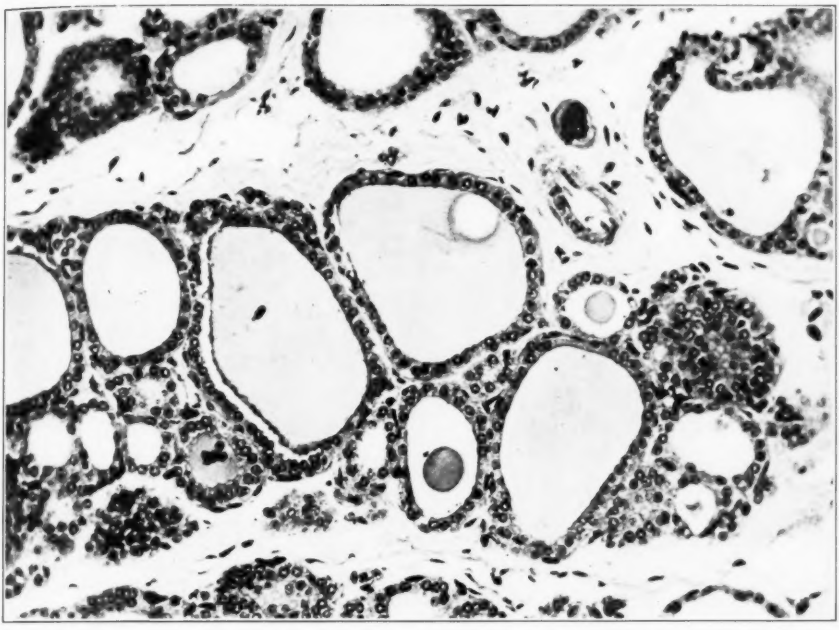
PLATE 157

FIG. 10. Thyroid from a girl 9 years old who died suddenly during recovery from diphtheria. Stage of restoration of the colloid. The old, denser colloid can be seen in several follicles. Uniform aspect of the epithelial lining of the distended follicles (cells with large nuclei — Type 3). × 300.

FIG. 11. Thyroid from man 21 years old. Fracture of the skull. Survival 48 hours. Specimen not fresh. Note the three excretory segments (columnar epithelium) belonging to three adjoining functional units, and the group of satellite follicles which lies under each columnar segment. × 230.



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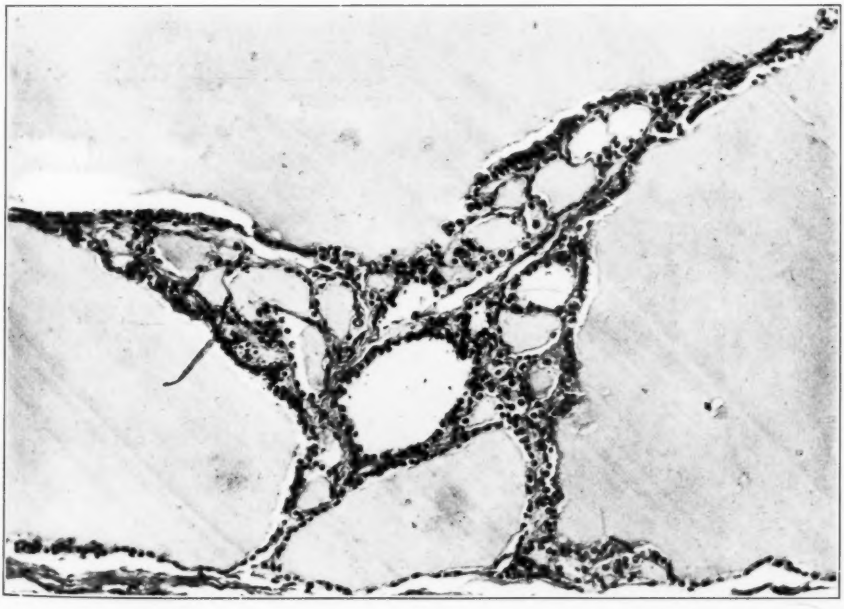

11

A UNIQUE INFECTION IN MAN CAUSED BY A NEW YEAST-LIKE ORGANISM, A PATHOGENIC MEMBER OF THE GENUS SEPEDONIUM *

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A middle-aged white man who had had an irretractable skin disease for 15 years attracted our attention because the extent and the gross features of the lesions were unlike anything we had ever observed. A survey of the literature convinced us that the clinical manifestations of the ailment indicated a new disease. Unique papular lesions with a small crater of necrosis capping each, which yielded a few drops of sticky pus, covered the entire skin. The lesions were heavily set on a much thickened, wrinkled and scaly skin, which had a tendency to become ulcerated. The ulcers were deep and crusted and progressed slowly. The regional lymph nodes were increased markedly in size. The features enumerated led us to believe that a moderately strong injurious agent was the cause of the disease. The extension of the lesions over the entire surface of the body, followed by enlargement of the regional lymph nodes, led us to suspect an infectious form of microbiological life, possibly a fungus, as the etiological agent of the disease. Sections of the pathological skin and lymph nodes revealed the presence of minute yeast-like bodies chiefly within endothelial phagocytes. The organism was isolated on artificial culture mediums. Mycological and animal pathogenicity studies of the pure culture were made. According to the taxonomy of Saccardo, the organism belonged to the genus *Sepedonium*. Our species did not belong to any of the described members of the genus. No pathogenic member of the *Sepedonium* has been described.

REPORT OF CASE

Clinical History: H. J., a steel welder, was observed on Feb. 9, 1931. He complained of a generalized skin eruption which was accompanied by intense itching. In 1917 dry scaly skin lesions developed in the regions of the popliteal

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spaces from which the remainder of the skin eventually became involved. He had been a sailor for 3 years, a railroad switchman for several years, and a welder of steel flues for the past 14 years. No flux or brass was used and the shop in which he worked was well ventilated. In September, 1929, lesions began to appear on the thighs. Progression of the lesions continued until he was unable to work because of involvement of the palms of the hands.

Physical examination revealed a man whose general condition was quite good. He had a generalized, dry, papular skin eruption which caused him considerable distress from itching. One buccal lesion was observed. Many types of local and general therapeutic agents were tried but these served only to relieve symptoms and had no effect upon the progress of the disease. He was discharged on March 29, 1931, with a diagnosis of dermatitis exfoliativa.

The patient was rehospitalized on July 7, 1932. Since his last hospitalization, ending on March 29, 1931, he had visited numerous physicians and health resorts but had received no aid. He had received a course of eight X-ray treatments in September, 1931, with no benefit, and a biopsy of the skin and an inguinal lymph gland was made in the institution where he had received the X-ray treatments. A diagnosis of dermatitis and lymphadenitis was made, but because of the heavy infiltration of the skin with lymphoid cells the question of leukemia of the skin was raised. Until May, 1932, the skin of his back was fairly smooth but since then the old lesions became more elevated and many new papules appeared. This was accompanied by loss of weight and weakness. He had difficulty in keeping warm even during the summer months. The right small toe became gangrenous and sloughed off in July, 1931. Several fingers of the right hand had been lost in an accident.

Physical examination revealed a weak, emaciated male, whose entire skin was involved in an extensive dermatitis. The lesions varied from scaliness and papule formation to ulcerated lesions measuring 3 to 4 cm. in diameter. The most recent lesions were papules measuring 0.5 to 1 cm. in diameter, which were somewhat irregular in outline and had a tendency to become confluent. The pruritus was intense. The thickened mucous membrane of the mouth presented several small, granular, ulcerated lesions. The heart, lungs and abdominal viscera were normal.

Laboratory Studies: The urine was normal. Red blood cells 4,200,000; white blood cells 10,200; hemoglobin 82. Blood Wassermann negative. Blood studies for evidence of changes in the bleeding time, coagulation time, fragility of the red blood cells, clot retractibility and prothrombin time showed no abnormalities. The blood platelets were 0.35 per cent (Van Allen). The CO_2 combining power of the plasma was 60.7. No lesions were demonstrable roentgenologically in the chest, or dorsal or lumbar vertebrae. Biopsies of the skin, the left inguinal lymph gland and the buccal mucosa were made on July 9, 1932. Each of these tissues was cultured and the yeast-like organism isolated. The tissue sections revealed definite evidence of a chronic inflammatory process and the presence of numerous yeast-like bodies in phagocytes. A few were free in the tissue and in the epithelial cells.

Therapy consisted of massive doses of potassium iodide and ionized copper treatment. X-ray was also applied to a small area of the lesions, all of which seemed to progress under treatment.

Under observation the patient developed new skin lesions and a punched-out ulcer of the tongue. Many of the older firm papules became ulcerated. His temperature, which had been of a mild septic type since admission, gradually

became more elevated. Three days before death friction rubs and râles were heard in the chest. Death occurred Aug. 7, 1932, presumably 15 years after the onset of the disease.

Postmortem Examination

The body was that of a well developed, emaciated male, whose entire skin was thickly studded with papular and macular lesions measuring 0.5 to 1 cm. in diameter. Many deep ulcers from 1 to 4 cm. in diameter were found, as well as numerous shallow ulcerations which represented the recent liquefaction of the summits of papules. Large decubitus ulcers were present over the sacral and scapular regions. The skin was thickened, reddish purple in color and fissured in many areas. No areas of the skin escaped involvement. The lesions were found in the scalp, eyebrows, palms of the hands, soles of the feet and the scrotal sac, as well as over the larger skin surfaces. Papular and ulcerated lesions were also found on the roof of the mouth, the tongue and the mucous membranes of the cheek.

Except for fibrous adhesions between the gall-bladder and the colon, the peritoneal cavity appeared normal. The left pleural cavity contained 150 cc. of cloudy sanguineous fluid. Fibrin covered the pleura. The right pleural cavity appeared normal. The lower lobes of both lungs were firm and not crepitant. The cut surfaces were granular and yielded a purulent fluid. The medium sized vessels were occluded by red, friable blood clots. The heart weighed 360 gm. No endocardial lesions were present. The spleen appeared somewhat fibrotic. The liver weighed 2670 gm. The cut surface had a yellowish color and yielded an abundance of greasy material. The gastro-intestinal tract, gall-bladder, pancreas and kidneys appeared normal. The adrenals were somewhat enlarged and showed small areas of necrosis in the medulla. Numerous atheromas and ulcerations were present on the intima of the aorta. The bladder and prostate appeared normal. The brain showed no lesions. All superficial lymph nodes were firm and enlarged, the largest being about 4 cm. in diameter.

Histological Examination

Except for slight myocardial scarring, the heart appeared normal. Sections obtained from the lower lobes of the lungs showed the alveolar sacs filled with polymorphonuclear leukocytes, fibrin and red blood cells. Many bronchioles showed partial or complete destruc-

tion of their walls. Several of the smaller branches of the pulmonary artery were occluded by organizing thrombi which showed canalization at their peripheries. Both old and recent infarcts were present. Occasionally yeast-like bodies were found which were engulfed by large mononuclear phagocytes. These organisms were found in the alveolar sacs and not in well defined lesions, as observed in the skin and the adrenals. The yeast-like bodies were not numerous. A few spiculated forms of the infectious agent were present. The spleen showed some thickening of the sinusoidal walls. The malpighian corpuscles were atrophic and many plasma cells were noted throughout the pulp. No lesions due to the presence of the organism were noted. The pancreas and gall-bladder appeared normal. The liver showed a moderate fibrosis and a chronic inflammatory cell infiltration of the supporting connective tissue of the portal canals. Fatty metamorphosis of the periphery of the lobules was present and a few small areas of focal necrosis were noted. The kidneys, except for a few small arteriosclerotic infarcts, appeared normal. The adrenals presented medullary and cortical areas of caseation necrosis resembling those produced by *Mycobacterium tuberculosis*. A few yeast-like bodies were present in the central portion of these caseous areas but they were found in great abundance in the phagocytes and the adrenal cells at the periphery. Myriads of microörganisms were found in the adrenal cells proper, in areas where no necrosis had as yet occurred. This was a conspicuous finding in all the early lesions. The largest adrenal lesion measured about 0.2 cm. in diameter. Large subintimal deposits of atheromatous material were observed in the aorta. Organizing thrombi were attached to the bed of some of the atheromatous ulcers. The skin sections taken before and after death, as well as the sections taken at another institution in September, 1931, revealed large numbers of microörganisms engulfed by large mononuclear leukocytes. A papule consisted of a collection of large mononuclear phagocytes located directly beneath the epidermis and confined to the corium. The infectious agent caused very little connective tissue proliferation. The larger, non-ulcerated lesions showed only slight evidence of liquefaction necrosis. Up to twenty or twenty-five rounded organisms, each surrounded by a distinct capsule, were contained within one phagocyte. A few organisms were noted in the epithelial cells themselves. Sections of the lymph glands, which were obtained before and after death, as well as the one removed elsewhere in September 1931, also revealed large

numbers of organisms. Scarring distorted the architecture of the glands so that no evidence of germinal centers was observed. Large numbers of plasma cells were present and a hyperplasia of the endothelial cells was in evidence. The latter contained the organisms. In a few instances collections of phagocytes heavily laden with yeast-like bodies were observed but as a rule they were scattered diffusely throughout the gland. Giant cells were abundant in the lymph glands, as compared to the other tissues involved, but they were not a prominent feature in any of the tissues. They contained relatively few organisms. The brain sections revealed no pathological changes.

Mycological Study

The appearance of the organisms in the tissues was that of rounded, yeast-like bodies. They varied little in size, and together with the chitinous fungus-cellulose capsule measured from 3 to 5 microns in diameter. There were a few swollen hyaline bodies which were judged to be empty capsules. The organisms were, for the most part, enclosed in monocytes but many were present in adrenal cells proper. A few were free in the tissues and some were present in epithelial cells of the epidermis and in giant cells. They were very numerous in injured adrenal cells at the periphery of a lesion. The organism stained better with various modifications of hematoxylin, (phosphotungstic acid hematoxylin and iron hematoxylin) than they did by the Giemsa method. In the lungs the organisms were larger than they were in the lymph nodes, the skin and the adrenals, measuring about 6 microns in diameter. Some of the yeast-like bodies in the lungs had a thick spiculated capsule, such as was later seen on artificial culture medium.

A biopsy of skin and lymph node was made on July 21, 1932. The tissue from each site was divided into two equal portions. One portion was washed in 6 changes of sterile broth. The other half was first treated momentarily with alcohol and then washed in 6 changes of sterile broth. The pieces of tissue were then macerated in a small amount of broth and test tubes of medium were then inoculated. What remained of the macerated tissue after inoculation of artificial mediums was inoculated into the peritoneal cavities of guinea pigs and mice. No evidence of disease developed in the animals after several months of observation.

Meat infusion agar, blood agar, brain agar, chocolate agar, Sabouraud's medium, 25 per cent rabbit blood agar, and beer-wort

agar were employed. The inoculum of a single loop was passed down a series of several large tubes of medium. The number of contaminating organisms was thus diminished so that isolated colonies of the fungus were readily obtained. In 7 days, there being no particular food requirement for the organism, numerous, small, flat, arborescent, icy-appearing colonies were barely visible on many of the tubes. At times there was no contamination, the tube being thickly set with colonies of the fungus. Even before the colonies appeared, smears from the surface of the medium revealed mycelial threads springing from the yeast-like bodies in a phagocyte. There seemed to be little difference in the rapidity of growth between the temperature range of 22° to 38° C. Under anaerobic conditions the growth was retarded. Colonies appeared first regularly on meat infusion and blood agar. On these mediums mycelial threads were more abundant. This was particularly true of the meat infusion agar. The growth on the mediums with a high percentage of serum and the mediums cultivated under anaerobic conditions developed more slowly, was butter-like in consistence and was composed almost entirely of large, round, yeast-like bodies.

Hanging drop cultures showed branching septate mycelium with the development of a large spore within a long mycelial thread, but much more frequently these spores were found within and at the end of a short mycelial branch. The surface of the spore was at first smooth, but later it took on a distinct spiculated appearance. Conidiospores were observed and there was no dissemination, indicating that the spores were well contained within the mycelial threads. Round, hyaline bodies, which were quite uniform in size and appeared as spores, were frequently seen within these large chlamydospores. They disappeared upon heating and absorbed fuchsin and sudan III. We were convinced of their lipoidal nature. These globules were especially abundant in cultures containing serum or those grown under anaerobic conditions. None of the globules survived fixation and staining methods.

Colonies grown directly on cover glasses showed a thallus of delicate mycelium. The flexibility of the filament is interpreted by the way its direction of growth is diverted when it encounters even the smallest particle on the cover slip. When a mycelium branches, the angle formed between the two mycelial threads is usually more than 45°. Usually after 10 days the large spiculated chlamydospores develop within and at the ends of the short lateral branches near the

center of the thallus. These spores extend peripherally as the thallus grows. After 3 weeks spores are about all that is left of the thallus. The mycelia are for the most part degenerated.

The mycological study in our case included a consideration of the various organisms which appear in tissue as yeast-like bodies. Leishman-Donovan bodies, Darling's *Histoplasma capsulatum*, *Oidium gilchristii*, *Monilia albicans*, *Coccidioides immitis*, *Torula histolytica*, *Phialophera verrucosa*, and the organism of pseudofarcy were compared with our organism. It appeared somewhat like Leishman-Donovan bodies in tissue. Giemsa's stain failed to bring out a kinetic nucleus. Unlike the organism of leishmaniasis, it was not pear-shaped and the chitinous cellulose material about the nuclear substance indicated a fungus rather than an animal parasite. The size of the organism in our case suggested the *Histoplasma capsulatum* of Darling, more than any of the above mentioned yeast-like organisms. It was much smaller than the other yeast-like bodies which appear in tissue. However, in the case here reported, there was no involvement of the spleen and the organism was culturally unlike the organism of pseudofarcy which has been presumed to be similar to Darling's *Histoplasma capsulatum*. The cultural characteristics were also quite unlike any of the above named organisms which appear in tissue as yeast-like bodies.

Animal Pathogenicity

Inoculation of macerated skin and lymph nodes into the peritoneal cavity of mice and guinea pigs produced no disease. The inoculation of guinea pigs and rabbits subcutaneously with the isolated fungus resulted in local lesions after 7 days. The lesions progressed for 7 days, when definitive evidence of regression was noted. The animals were killed after approximately 4 weeks. The organism was still alive in the lesion but there was no dissemination of the infection and it seemed definite that the lesions would have healed in these animals. The dog and the rat developed progressive lesions. The animals were killed, but judging from the extensive lesions in the lungs, spleen, adrenals and liver, it appeared relatively certain that these animals would have died of their generalized infection. The small yeast-like bodies were found in the granulomatous lesions of these experimental animals. Pure cultures of the yeast-like organism were isolated from the lesions 3 to 4 weeks after the inoculation of the animal.

Summary and Conclusions

A case of a chronic infection produced by a yeast-like organism belonging to the genus *Sepedonium* has been reported. The infectious agent was apparently localized in the skin and the regional lymph nodes for a period of about 15 years. The skin was thickened and scaly throughout the course of the disease, except during the last 3 months of life when the characteristic papular lesions developed. It is possible that this fungus infection could have been a secondary infection ingrafted upon a non-specific scaly dermatitis, but the presence of the yeast-like organism in the skin and lymph glands for at least a year and a half before the lesions became papular, and the fact that the enlargement of the lymph nodes was an early observation, make this possibility seem quite improbable. It is our opinion that the disease was initiated by the fungus.

The appearance of the organism in tissue, the large spiculated chlamydospores on artificial culture medium and the animal pathogenicity of the organism are the characteristic features by which subsequent cases may be recognized.

The infecting organism is similar in the chronicity of the infection it produced, the macroscopic appearance of its growth upon artificial culture medium and the formation of spores upon lateral branches to the so-called oidium mentioned in medical literature. However, the large spiculated spores, the delicate mycelium and the animal pathogenicity are distinctly different from the *Oidium gilchristii*.

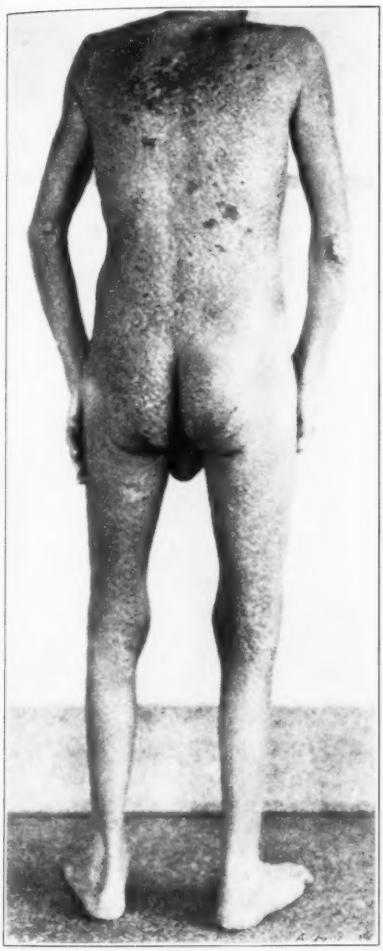
Although we appreciate that the taxonomy of this large group of imperfect fungi, to which this organism belongs, is artificial and often very unsatisfactory, it would appear that this organism could not be more satisfactorily classified for the present than with the genus *Sepedonium*, since no spore formation from the copulation of hyphae was observed.

DESCRIPTION OF PLATES

Plate 158

Figs. 1 and 2. Photographs showing the distribution and nature of the skin lesions.

Fig. 3. Photograph of the thickened, wrinkled, scaly skin which shows many papules with crater-like ulcerations of their summits.



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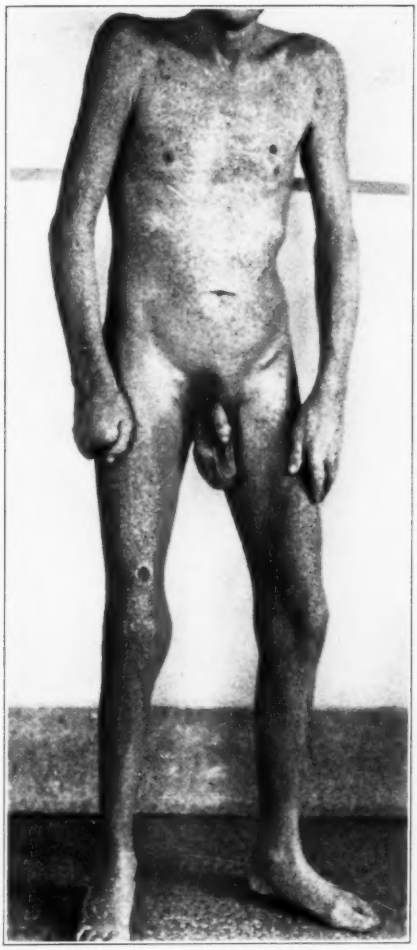


2

3

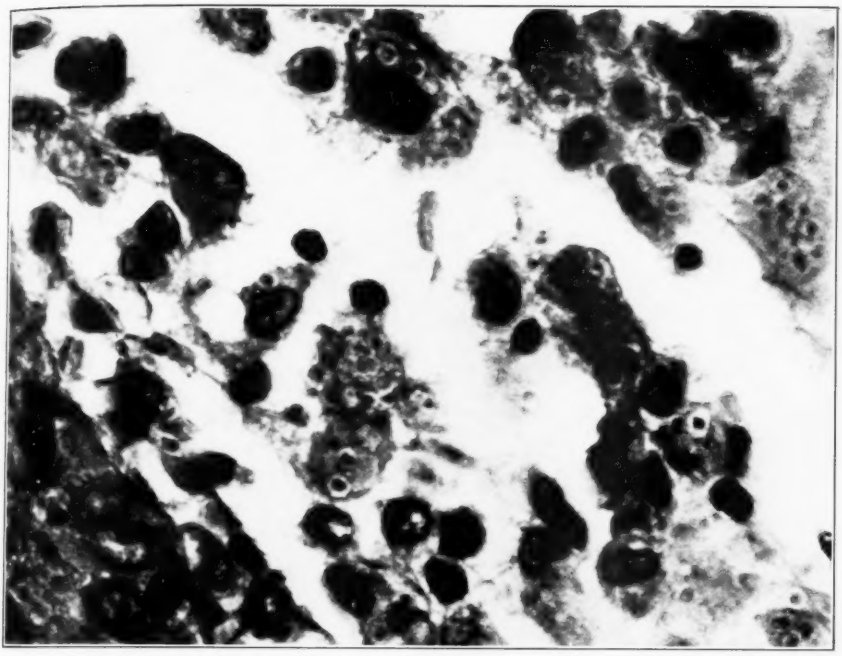
Hansmann and Schenken

Infection Caused by New Yeast-Like Organism

PLATE 159

FIG. 4. Photomicrograph of the yeast-like organism in the large mononuclear cells in the corium of the skin. × 1200.

FIG. 5. Photomicrograph showing the large, thick-walled, spiculated chlamydospores which are so characteristic of the organism upon artificial culture medium. × 1200.



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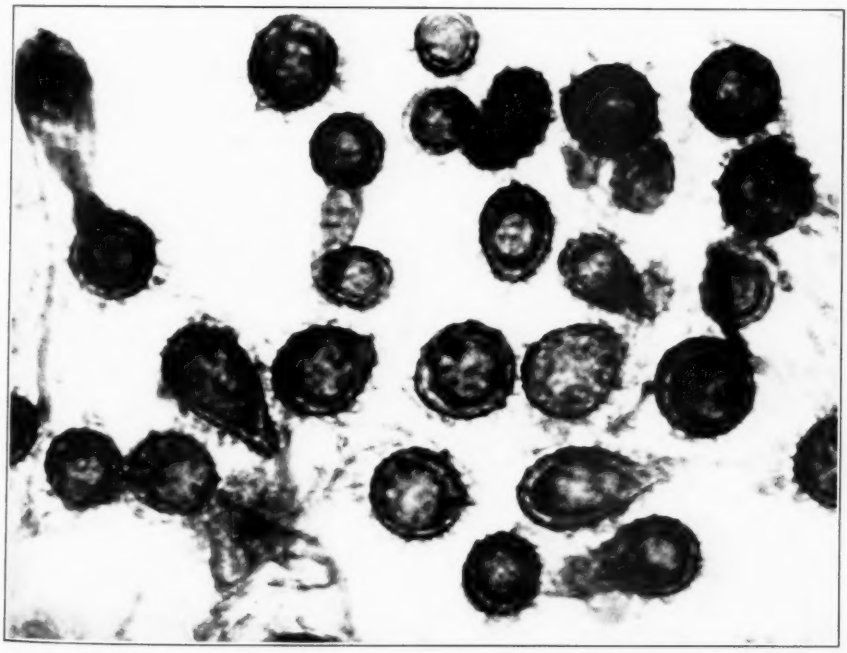

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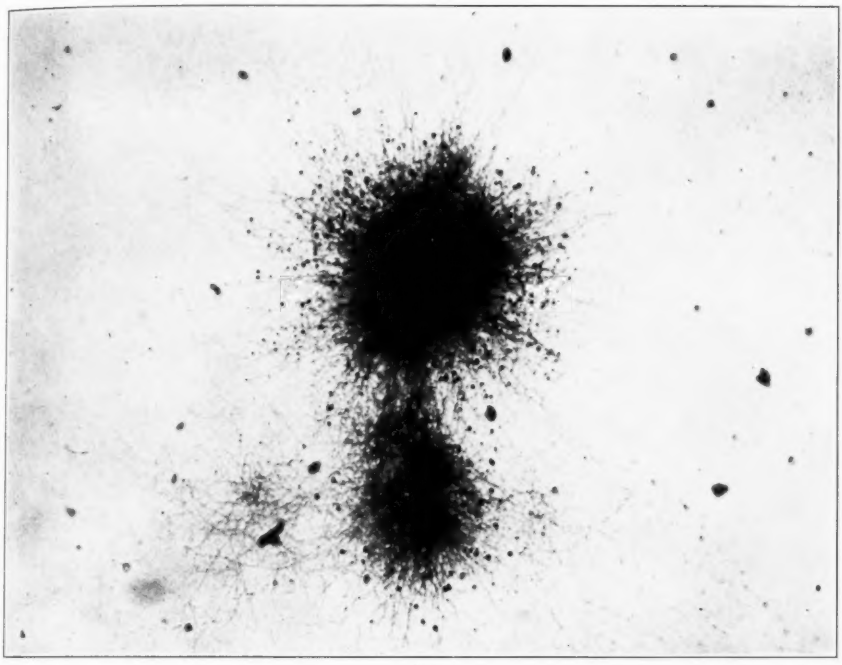
Hansmann and Schenken Infection Caused by New Yeast-Like Organism

PLATE 160

FIG. 6. Photomicrograph of a thallus of the organism grown on a glass slide. Note the large spores near the center of the thallus and the delicate, tangled mycelium. × 60.

FIG. 7. Photomicrograph of the adrenal of the human showing an area of caseation necrosis in which many organisms were found. × 70.



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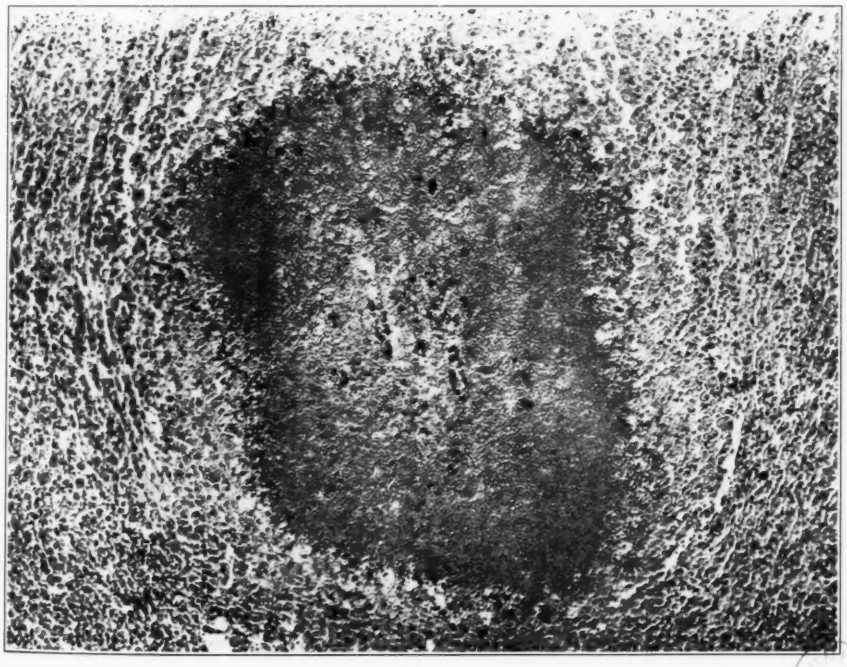

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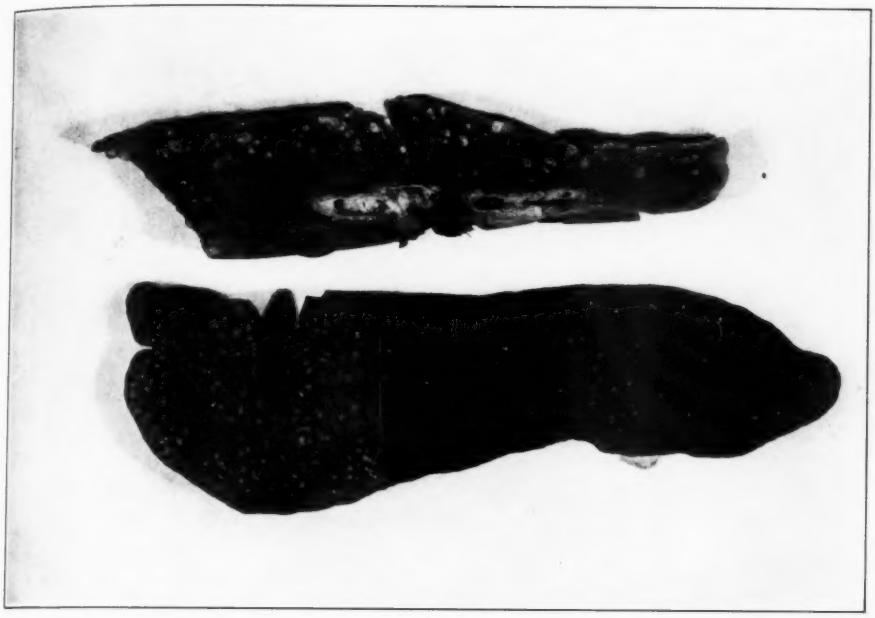
PLATE 161

FIG. 8. Photograph of the lesions in the lung and the spleen of the dog inoculated with the organism.

FIG. 9. Photomicrograph of the lung of a dog showing a granulomatous lesion of the disease. × 60.



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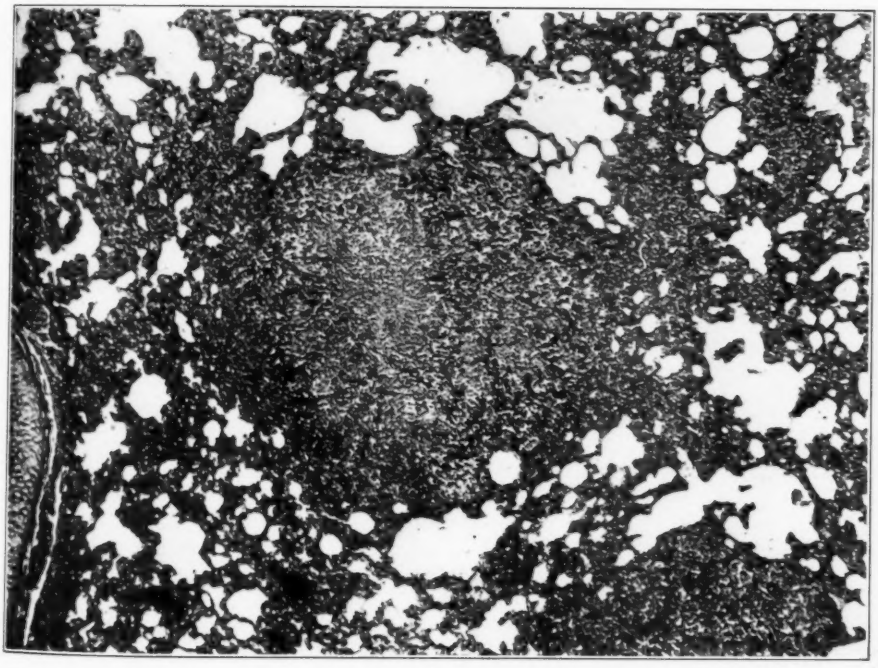

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Hansmann and Schenken Infection Caused by New Yeast-Like Organism

A FREE GROWTH PERIOD OF TUBERCLE BACILLI IN THE GUINEA PIG OMENTUM AS RELATED TO THE HYPERSENSITIVE STATE *

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The study of immune processes in tuberculosis is complicated by the fact that true immunity, in the sense in which that term is used in diphtheria or smallpox, does not exist. The patient who, to all clinical appearances, has completely recovered from tuberculosis may suffer a relapse, just as the experimental animal which has been "immunized" may be fatally reinfected with a large enough dose of virulent tubercle bacilli. At the same time, as was first adequately demonstrated by Römer,[1] something happens, in the laboratory animal and, presumably, in man, after an initial tuberculous infection, which renders the individual or the animal somewhat more resistant to reinfection. Just what changes occur in the body cells and fluids following this initial sensitizing infection is not clear.

A useful approach to the problem of immunity in tuberculosis and one which has been employed by numerous investigators, is the attempt to discover how the tubercle bacillus itself, with its resistant, waxy structure, is affected by inoculation in the normal and the "immune" animal. Thus Markl,[2] Kraus and Hofer,[3] Manwaring and Bronfenbrenner,[4] Bergel,[5] Rist, Léon-Kindberg and Rolland [6] and Dworski, Smith and Gardner [7] all have studied peritoneal fluid withdrawn at intervals after intraperitoneal inoculation of the experimental animal, and Paterson [8] has made a similar study of pleural exudates. All of these studies, however, are subject to the limitations imposed by the use of inflammatory exudates, most of the investigators having noted the early appearance of so-called Much's granules and the complete disappearance of acid-fast bacilli from the fluid of the respective serous cavities within 4 or 5 days after inoculation. In every case the bacilli were found to have disappeared more quickly in the reinoculated than in the normal animal.

* Received for publication July 9, 1934.

In the study which follows use has been made of the well known fact that bacteria, when inoculated in the peritoneal cavity, accumulate in large part in the omentum. Spread preparations of the omentum have been employed, a simple technic having been developed for staining such preparations. This use of omental spreads has made it possible to study the inoculated bacilli in their normal relation to the fixed tissue cells and the developing tubercles, a study that cannot be made adequately either with histological sections or with smears of the body fluids.

Technic

Albino guinea pigs were employed in the major part of the experiments, since the reaction of this animal to tuberculous infection seems to approximate so closely that of man. In general, young animals from 200 to 300 gm. in weight were used, as the omentums of old guinea pigs contain too much perivascular fat to make good spread preparations.

In most of the experiments the well known H-37 human strain of *Mycobacterium tuberculosis* was used for the inoculum. A portion of the pellicle of a glycerine broth culture was weighed, after blotting off the excess fluid on sterile filter paper, and carefully ground with mortar and pestle in a few drops of sterile saline. Saline was then slowly added to give the dilution which was desired, usually 1 mg. of the culture per cubic centimeter of fluid.

Anyone who has attempted to prepare inoculums from cultures of the tubercle bacillus has experienced the difficulty of getting a uniform suspension of the organisms. Even after the most painstaking grinding relatively large clumps of bacilli remain in the triturated material. In the present experiments no attempt was made to remove these clumps by filtration since it was desired to maintain the known weight of inoculum. In most of the experiments the animals were given an amount of inoculum equivalent to 0.1 mg. of bacilli per 100 gm. of body weight, though in some series as much as five times this amount was employed. Whenever inoculums containing clumps of bacilli were employed, it was found important to take into the syringe sufficient inoculating fluid for only one animal at a time. If enough fluid for several animals is taken into the syringe the clumps of bacilli gravitate so rapidly that an uneven dose for the different animals results.

Another pitfall which must be guarded against in making intraperitoneal inoculations is that of losing all or a portion of the inoculum either by penetrating the bowel or by failing to enter the peritoneal cavity at all. In the present study the animals, with abdomen shaved, were lightly etherized and an incision 3 to 4 mm. in length made through the skin with scissors. By making use of this incision one could usually be certain of entering the peritoneal cavity with the inoculating needle. The best protection against penetration of the gut we have found to be an initial inoculation of 10 cc. of air with a separate syringe. If the air enters the peritoneal cavity the anterior abdominal wall will be bulged up uniformly, while if the air is forced into the appendix or some other portion of the gut a serpentine bulge is formed. Once the anterior abdominal wall has been separated from the underlying intestines by a layer of air the inoculating needle can be introduced with little fear of penetrating the bowel. If the inoculating dose should be lost in its entirety into the gut the omentum remains completely normal.

Omental Spreads

In order to follow the omental changes animals were killed at daily, or more frequent, intervals. During the first few days after inoculation omental spreads are easily made. After the first week, however, there is an increasing tendency for the free edges of the omentum to become matted together. Under these circumstances it is well to pull with forceps on the matted portion of omentum before it is removed from the animal, thus breaking most of the fibrinous adhesions. That part of the omentum which shows the maximum involvement is then freed with scissors and mounted on a clean glass slide, either directly or after first floating the tissue in a jar of normal saline. Using a pointed glass rod for manipulating the moist preparation, a single, thin layer of the omentum is isolated and stretched out from the major omental mass. By allowing this thin film of tissue to dry on the slide one obtains a point of attachment and can pull from this point on the main mass of omentum further to break apart its fibrinous adhesions. Finally one obtains a layer of omentum not more than one cell thick through most of its extent. The preparation is then allowed to dry in the air, after which it will be found firmly fixed to the slide. The steaming with carbol fuchsin completes the fixing process.

With minor exceptions the ordinary Ziehl-Neelsen technic for staining is employed. The preparation, flooded with carbol fuchsin, is heated to the steaming point on an aluminum plate, washed in tap water, flooded with acid alcohol, washed, and then immersed for 10 minutes in acid alcohol (3 per cent HCl in 95 per cent alcohol) in an attempt to decolorize the thicker portions of the preparation. After again washing in tap water the omentum is counterstained with methylene blue, washed and blotted. The preparation should then be allowed to dry for an hour at 37°C or for 12 hours at room temperature to allow the perivascular fat to "sweat out." Finally, any remaining portions which are too thick should be removed. The process is completed by merely immersing the dry preparation in absolute alcohol, then in xylol, and mounting in balsam. The result is a permanent preparation, easily and quickly available, which shows the entire developing tubercle with its content of bacilli and its surrounding cells. Such a preparation may be studied through most of its extent with the oil immersion lens.

Results

I. Inoculation of the Normal Guinea Pig

The results which we have obtained in the early hours after intraperitoneal inoculation corroborate those reported by other workers. There is an initial acute inflammatory reaction, the tubercle bacillus being taken up first by polymorphonuclear cells. One of the surprising findings was the rapid disappearance of the large clumps of bacilli (Fig. 1) which were included in the inoculum. A few hours after inoculation these clumps had been almost completely broken up by the polymorphonuclears, each cell taking up from one to a dozen bacilli (Figs. 2 and 3), and by the end of 24 hours the clumps had completely disappeared. At 2 hours there are relatively few large mononuclear leukocytes, but an occasional one will be found which has phagocytosed bacilli. In no case, however, have we found the mononuclear cells phagocytosing polymorphonuclears at this stage. Unfortunately, the Ziehl-Neelsen stain used for our preparations did not enable us to differentiate between polymorphonuclear neutrophiles and eosinophiles, the latter, according to Dworski, Smith and Gardner,[7] being the cell most actively phagocytic for the tubercle bacillus in the first few minutes after inoculation.

After 24 hours the peritoneal fluid and the omentum still show numerous polymorphonuclear leukocytes, but very few of these cells contain tubercle bacilli. Many of the polymorphonuclears which phagocytosed bacilli during the first few hours have now been taken up by large mononuclear cells (Figs. 4 and 5).* The bacilli, as seen after this double phagocytosis, exhibit marked irregularities in staining and in many cells non-acid-fast granules may be seen which probably represent the so-called Much's granules. Also, occasional mononuclear cells are seen which have evidently taken up bacilli directly (Fig. 6). It will be noted that these bacilli appear to lie within cytoplasmic vacuoles. Furthermore, they appear better preserved than the bacilli in Figures 4 and 5 which were first phagocytosed by polymorphonuclears.

While many damaged polymorphonuclear leukocytes are taken up by large mononuclear cells, as illustrated in Figures 4 and 5, a large portion of the polymorphonuclears bearing tubercle bacilli apparently gravitates to the milk spots (*tache laiteuse*) of the omentum or to the perivascular fat tissue. The dark areas shown about the blood vessel in Figure 7 are made up almost entirely of polymorphonuclears which have encroached upon the perivascular fat cells. Many of these polymorphonuclears still carry their burden of bacilli. Within 3 days after inoculation the perivascular areas have become a dense cellular mass composed largely of mononuclear cells (Fig. 8). It is the opinion of Gardner,[10] who has studied this phenomenon in regular histological sections, that the mononuclear cells proliferate *in situ* to form the tubercle-like nodules about the blood vessels. It is in these areas that caseation first appears.

The Appearance of Freely Growing Bacilli: Gardner [11] has pointed out the remarkable clearing of the acute inflammatory reaction within 3 days after intraperitoneal inoculation of the normal guinea pig with tubercle bacilli. We have observed the same thing in spread preparations of the omentum. At 4 days the preparations show the dense perivascular infiltration, the enlarged milk spots and occasional clumps of mononuclear cells which apparently proliferate *in situ* on the surface of the omentum to form miliary tubercles (Fig. 9). At 4 days, also, a phenomenon which we have not hitherto

* A recent paper by Vorwald [9] indicates a similar transference of bacillus-containing polymorphonuclears to the large mononuclear cells in the lung of the rabbit after intravenous inoculation with H-37.

seen reported makes its appearance. In association with certain cells may be seen masses of bacilli growing in parallel strands just as they might grow on the surface of glycerine broth (Figs. 10 and 11). These masses may be immediately adjacent to, or some little distance from, the groups of cells that are forming tubercles (Figs. 10, 11, 12 and 13) and, surprisingly enough, show no sign whatever at this stage of attracting inflammatory cells of any type (Figs. 14 and 15). The bacilli commonly follow the cytoplasmic outline of the cell with which they are associated and at times are looped about the cell nucleus (Fig. 16). However, they appear to be growing on the surface of, rather than within, the cytoplasm. From all appearances the cells in which, or on which, the bacilli are growing are the ordinary ones which make up the major portion of the omental network (Figs. 17 and 18). They show no deleterious effect from the symbiotic growth. These organisms, because of the capacity which they exhibit for growth without the least interference from either the cells or body fluids of the animal in which they are proliferating, have been termed *freely growing bacilli.*

That the microörganisms which have just been described have actually proliferated and do not represent merely clumps of bacilli from the inoculum is indicated, first, by the fact that no such clumps are found in the omentum in the interim between inoculation and the 4th day and that all large clumps have disappeared from the peritoneal fluid by the end of 24 hours; secondly by the fact that the bacilli are in parallel strands just as they grow on culture media, while the inoculated bacilli, after phagocytosis by either polymorphonuclears or monocytes, are found helter-skelter in the cell without any regular arrangement. Thirdly, only a few small colonies of freely growing bacilli are found in an occasional omentum at the 4th day, while, on the 6th day the colonies are larger and more numerous and are found with uniformity in the omentums of most of the animals. Fourthly, the freely growing forms do not appear in the omentum of "immunized" or secondarily inoculated guinea pigs. Finally, various control inoculations (to be described in detail later) made with caseous material, heat-killed bacilli, and timothy grass bacilli prove that the tubercle bacillus does actually proliferate in the animal body at a certain period after inoculation without any inflammatory response on the part of the diseased animal. The great regularity with which the free proliferation of tubercle bacilli

occurs in the body of the normal guinea pig is indicated by the accompanying table.

TABLE I

Normal Guinea Pigs Inoculated with H-37 from Glycerine Broth Cultures *

Days after inoculation	1	2	3	4	5	6	7	8	9	10	11	12	13–78
Total No. pigs sacrificed	24	13	12	15	15	26	18	18	14	11	13	12	103
No. of pigs *positive* for free bacilli ..	0	0	0	6	11	21	16	6	2	1	1	0	0
No. of pigs *negative* for free bacilli ..	24	13	12	9	4	5	2	12	12	10	12	12	103

* Based upon 21 series of guinea pig inoculations.

As indicated, the freely growing forms were found first after an interval of 4 days. Four days is about the length of time required for the H-37 strain to establish itself on favorable culture mediums and this fact probably explains why freely growing bacilli were not found before the 4th day after inoculation. From the 4th day the number of animals showing the freely growing bacilli rises to 81 per cent at 6 days* and 89 per cent at 7 days, falling off abruptly after the 7th day.

The Use of Caseous Material as Inoculum: Five series of animals were inoculated with caseous material obtained from cold abscesses, the latter being the result of inoculating guinea pigs subcutaneously with H-37. This caseous material can be readily triturated and diluted with saline to give a uniform suspension. The amount of inoculum used varied from 10 mg. of caseous material per pig in 1 series to a maximum of 50 mg. per pig in another series. Smears of the inoculum showed only scattered acid-fast bacilli, while in 2 series the caseous material contained no demonstrable acid-fast organisms whatsoever. In every series, however, masses of freely growing tubercle bacilli made their appearance, Table II presenting the accumulated data from the 5 series.

* A careful reading of one of Krause's [12] early papers indicates that he must have observed freely growing bacilli in the iliac lymph nodes of guinea pigs after subcutaneous inoculation in the groin. In describing the masses of bacilli which have proliferated in these nodes at 6 days Krause says, "In none of these particular fields did the bacilli appear to lie within the cells. . . ."

It will be noted from Table II that the freely growing bacilli in these series were somewhat more tardy in making their appearance than when the inoculum was obtained from broth cultures. When they eventually appeared, however, the freely growing forms were perfectly typical in arrangement (Figs. 19 and 20).

TABLE II

Normal Guinea Pigs Inoculated with H-37 Caseous Material obtained from Cold Abscesses *

Days after inoculation	1	2	3	4	5	6	7	8	9	10	11	12	13–47
Total No. of pigs sacrificed	4	2	3	2	3	2	3	1	6	1	1	1	47
No. of pigs *positive* for free bacilli	0	0	0	0	0	2	3	1	2	0	0	1	0
No. of pigs *negative* for free bacilli	4	2	3	2	3	0	0	0	4	1	1	0	47

* Based upon 5 series of guinea pig inoculations.

A single series of animals was inoculated with the bovine strain, H-61, and another series with caseous material obtained at the autopsy table from a case of generalized tuberculosis in man. In both series beautiful examples of freely growing bacilli were found.

The Use of Dead Bacilli and of M. Phlei as Inoculum: Three series of pigs were inoculated intraperitoneally with H-37 killed by boiling and 1 series with the same organisms killed by exposure to direct sunlight. In the 4 series 26 guinea pigs were employed. Tubercles were formed in the omentums of these guinea pigs and, in the tubercles, faintly staining acid-fast bacilli were found for as long as 22 days after inoculation. Though the inoculums — in each case 1 mg. per pig — contained large clumps of bacilli, in no instance was anything found in the omentum suggestive of the freely growing form of the bacillus.

In 4 series, utilizing 33 pigs, the timothy grass bacillus, *M. phlei*, was employed as inoculum. The relatively small lipoid content of this bacillus, as compared with the pathogenic acid-fast strains, has been pointed out by Anderson,[13] a fact that may explain the early disappearance of the inoculated grass bacilli from the omental preparations. However, tubercles are formed sooner than after in-

oculation with H-37 (Figs. 21 and 22). Acid-fast bacilli can be found in these tubercles in large numbers during the first 24 hours, but by 3 days and thereafter bacilli can no longer be found. The tubercles do not progress to caseation.

II. The Inoculation of Hypersensitive Guinea Pigs and of Normal Rabbits

The results from the inoculation of hypersensitive pigs contrast strikingly with those obtained from the normal animals. In 3 series the pigs were given a subcutaneous sensitizing dose of H-37 from 3 to 5 weeks before the secondary intraperitoneal inoculation. As far as the exudative reaction in the hypersensitive animal is concerned our findings again corroborate those of Gardner.[11] The initial outpouring of polymorphonuclears is greater than in the normal animal. Tubercles form more rapidly, and the omentum, within 4 days after the second inoculation, is matted together in a dense sausage-shaped mass which cannot be separated without tearing the tissues. Within this matted omentum may be found accumulations of creamy caseo-pus, while a dense fibrinopurulent exudate is frequently found adhering to the surfaces or edges of the liver and spleen. If, at daily intervals, smears are made of either the pus or the fibrinopurulent exudate they will be seen to contain the same large clumps of acid-fast bacilli that were included in the original inoculum (Fig. 23). These clumps are closely surrounded by polymorphonuclear leukocytes, the cells being literally plastered against the edges of the bacillary mass. An occasional cell in the smear (either polymorphonuclear or mononuclear) may contain a few bacilli, but there is no sign of the breaking up of bacillary clumps through the phagocytosis which occurs in the normal pig.

At 10 days the clumps are still found, somewhat more rounded or oval in shape, with a ring of polymorphonuclears still pressing tightly against the bacilli (Figs. 24, 25 and 26). The latter appear to have undergone partial lysis, no longer staining as sharply as in the early days after inoculation. Thus, in the hypersensitive guinea pig the clumps of bacilli which may be included in the inoculum are not broken up by phagocytosis, as they are in the normal animal. Furthermore, at no time in the hypersensitive animals is the phenomenon of free proliferation of the inoculated tubercle bacilli ob-

served. In none of the 31 animals used in the 3 series was there any suggestion of that free growth of tubercle bacilli which occurs in the normal guinea pig.

Since the normal rabbit has a high degree of natural resistance to infection with the human type of tubercle bacillus, it was decided to inoculate some of these animals. Accordingly, a small group, 9 rabbits in all, was inoculated intraperitoneally with H-37 on the same basis as the guinea pigs, namely, 0.1 mg. of bacilli per 100 gm. of body weight. None of the inoculated rabbits showed at any time any free growth of tubercle bacilli.

III. The Fate of the Freely Growing Bacilli

Up to 6 days after inoculation the freely growing bacilli show no evidence of attracting phagocytic cells (Figs. 10 to 18), although by the 7th day they usually begin to exert a chemotactic influence upon the polymorphonuclear leukocytes. Frequently in a single omental preparation at this stage one finds a few phagocytic cells hovering about at a little distance from the bacilli (Figs. 27 and 28), while another colony of bacilli may have a polymorphonuclear directly applied to it (Fig. 29). Still another colony may be completely broken up by the invasion of polymorphonuclear leukocytes (Figs. 30 and 31). The bacilli at this period show a marked degree of beading (Fig. 32).

At 7 days, then, the omentum of a single animal may show considerable variation in the status of the masses of freely growing bacilli, though by the 8th day the freely growing forms have disappeared in the majority of the animals. In their place we find evidence of the formation of a secondary series of tubercles, and by 9 days this phenomenon is well established. At this time the omental preparations show two definite vintages, so to speak, of tubercles — large ones which have formed about the original bacilli of the inoculum, and minute ones which are in the process of forming about the once freely growing bacilli (Fig. 33). It is frequently impossible to make out the bacillary content of the large tubercles at this stage, even when they are teased apart, while in the minute tubercles bacilli are clearly visible (Fig. 34).

During the period from 10 to 14 days after inoculation one gains the impression that the number of acid-fast bacilli is definitely less,

occasional preparations showing beaded, non-acid-fast forms. Acid-fast bacilli, if found at this stage, are frequently bizarre in shape, as shown in Figure 35. At much later stages the omentum still shows acid-fast bacilli which are irregular in length and in staining reaction (Fig. 36). Some of these bacilli appear to lie free on the surface of cells, but there are no freely growing colonies such as characterize the 4 to 7 day interval. Any phagocytosis at this stage is apparently by cells of the mononuclear series (Fig. 36).

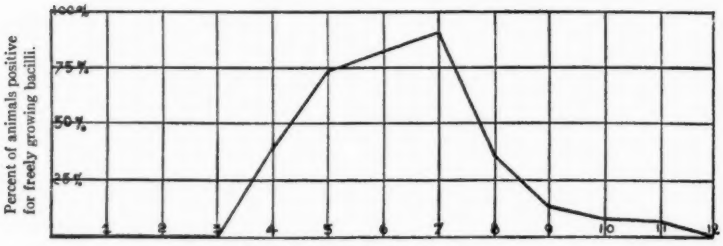
Discussion

From the data presented it is evident that tubercle bacilli of the H-37 strain, after intraperitoneal inoculation in the normal guinea pig, pass through certain definite changes, as far as their chemotactic relation to cells of the host animal is concerned. The inoculated bacilli attract and are taken up by polymorphonuclear leukocytes, which then either move to the perivascular tissues or milk spots, or are phagocytosed in turn by large mononuclear cells. At 4 to 6 days the freely growing forms appear and show no sign of chemotactic influence until the 7th day, when they again attract the polymorphonuclear cells. It seems probable that this variable picture, as regards chemotaxis, is due to one of two things — either to changes that occur in the cells and (or) fluids of the infected animal, or to changes that occur in the biochemistry of the inoculated microörganisms. In favor of the former hypothesis is the fact that hypersensitiveness, as indicated by a positive tuberculin reaction, appears within 6 to 12 days after experimental inoculation of the guinea pig with tubercle bacilli.[14] To determine whether there is any correlation between the appearance of a positive tuberculin reaction and the disappearance of freely growing bacilli in a given animal, a small series of pigs was tested by the Mantoux method. In this small series there was no absolute correlation observable, the freely growing bacilli having usually disappeared several days before the appearance of a positive tuberculin reaction. It was noted, however, that whenever the tuberculin test did become positive in an animal there was no longer any sign of freely growing bacilli in that animal's omentum.

In favor of the hypothesis that some change in the biochemistry of the bacillus is responsible for the chemotactic cycle is the abrupt

falling off in the percentage of animals positive for freely growing bacilli.

As indicated in the curve below, the proportion of animals showing the freely growing forms falls from 89 per cent at 7 days to 9 per cent at 10 days, a rather more abrupt drop than one would expect if the decrease were due merely to the gradual development of hypersensitiveness in the animals during the interval from 6 to 12 days. The abrupt appearance of freely growing bacilli at 4 days we have attributed to the latent period required for the H-37 strain to adjust



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itself to culture mediums of any type. Possibly the abrupt disappearance of these freely growing forms is again dependent upon some change in the characteristics of the bacilli themselves. Kahn [15] has demonstrated in beautiful manner that the H-37 strain of *M. tuberculosis* may pass through a complicated pleomorphic cycle when grown on certain culture mediums. The marked beading of the freely growing bacilli (Figs. 29, 30 and 32) is suggestive of the zoning within certain bacilli, which Kahn describes as occurring in his microdroplet cultures, or of the granules to which Gróh [16] attaches such significance.

It is impossible, at the present time, to decide definitely whether the abrupt reappearance of positive chemotaxis and the concomitant disappearance of the freely growing bacilli is due to the development of immune bodies in the infected animal or to changes in the bacillus itself, or to both. It seems certain, however, from our experience with the 31 secondarily inoculated animals, that the phase of free growth does not occur at all in an animal once it has been rendered hypersensitive. Thus, whatever else it may mean,

the positive tuberculin reaction in a guinea pig indicates that that animal is no longer susceptible to the type of free, unencumbered growth of tubercle bacilli, which takes place with uniformity in the normal animal.

Since we have noted that the freely growing bacilli disappear *before* the tuberculin test becomes positive, it seems possible, if not probable, that some reaction more delicate than the tuberculin test, which will indicate this changed condition of the animal, may be discovered. A further basis for this hypothesis is to be found in our results with the series of normal rabbits which failed to show free growth of the inoculated tubercle bacilli at any time. The normal rabbit does not, of course, exhibit a positive tuberculin reaction. It is interesting to speculate upon the possibility that the natural resistance of rabbits to infection with the human type of *M. tuberculosis* is related in some way to this failure of the free growth of the inoculated bacilli.

In the occurrence of the free growth of tubercle bacilli within the body of the normal guinea pig and the failure of that type of growth to occur in the hypersensitive animal we find the refutation of those workers who maintain that the difference between the reactions of the two types of animals is merely quantitative.[17] To be sure, the inflammatory reaction in the hypersensitive animal is quantitatively greater at the outset, but this reaction is maintained, instead of clearing up completely, as in the normal pig. This difference seems to us qualitative in nature, particularly when viewed in the light of the different effect upon the growth of the tubercle bacillus during the first week after inoculation. Rich,[18] in arguing against the acute inflammatory reaction of the allergic animal as a factor in "localization" of injected bacilli says: "Certainly, we ourselves have never been able to discover any difference between the number of stainable bacilli at the site of inoculation into the skins of normal and immune animals during the first few days after the inoculation; and after that, the number of bacilli in the area in the normal animals surpasses that in the immune animals — quite the reverse of what one would expect to find if the bacilli were quickly drained from the site in the normal animals, and actually held there bodily for days in the immune animals." Though the given data, particularly with regard to time intervals, are incomplete, it is our impression that Rich, in finding an increased number of organisms in his normal guinea pigs

after several days, was dealing with freely growing forms of the tubercle bacillus. In a series of normal animals which we inoculated subcutaneously, evidence was obtained that the free growth of tubercle bacilli occurs 6 or 7 days after subcutaneous inoculation, as well as after the intraperitoneal route of infection.*

An impression which inevitably follows working with omental preparations from several hundred infected guinea pigs is that one must have a very definite respect for the function of the polymorphonuclear leukocyte in the immune processes of tuberculosis. The polymorphonuclear has fallen into low esteem in this disease due, in part, to the well known fact that the opsonic index is lower in the animal rendered hypersensitive to tuberculosis than in the normal animal. To Zinsser [19] ". . . it is perfectly plain at the present time that polymorphonuclear phagocytosis has no protective value in tubercle bacillus infection. Indeed the tubercle bacilli are carried by the polynuclears throughout the body and any intra-cellular destruction that takes place is the function of clasmatocytes and giant cells."

While it is undoubtedly true that tubercle bacilli are disseminated by the polymorphonuclear leukocytes following a primary infection, it is also evident that the bacilli grow at a certain period in perfect symbiosis with the omental cells — cells of the connective tissue series which are, presumably, closely related to the clasmatocytes. Furthermore, it is the polymorphonuclears that first attack and break up the freely growing bacilli and initiate the process of renewed tubercle formation. Also, it is the polymorphonuclears that we find, in the hypersensitive pigs, plastered about the clumps of inoculated bacilli — not phagocytosing them but preventing the clump from breaking up, helping to localize the bacilli, as Krause and Willis [20] have shown and, we surmise, helping to prevent the free growth which occurs in the normal animal. This reaction of the polymorphonuclear cells in the hypersensitive animal is perfectly compatible with the existence of a low opsonic index, the low index, in fact, being an indication of the new function of the polymorphonuclear leukocyte in these animals, in which as Krause [21] says, "An almost immediate inflammatory outpouring hems in the bacilli more

* The possibility that these freely growing bacilli may represent an R dissociant has been considered, though no actual experimental work bearing on this point has been performed.

or less effectively and thus delays or prevents their spread which is so facile and rapid in the non-tuberculous, non-allergic animal."

We can agree with Rich's [18] conception of the local fixation of bacilli as a phenomenon "separate and dissociable" from allergy. At the same time, however, we believe that the polymorphonuclear leukocyte plays an important rôle in determining whether or not inoculated tubercle bacilli may grow freely for a certain period in the body of the host, and that this cell, therefore, is a factor which must be given due consideration in any discussion of immunity in tuberculosis.

Summary

1. After intraperitoneal inoculation in the guinea pig the H-37 strain of tubercle bacillus is first subject to phagocytosis by polymorphonuclear leukocytes. Then certain of the bacilli grow freely for a period on, or in, the cells of the omentum without exhibiting any chemotactic influence. At the end of this period the bacilli again attract polymorphonuclear leukocytes.

2. In guinea pigs that have been rendered hypersensitive to tuberculosis, and in normal rabbits, free growth of inoculated tubercle bacilli does not occur.

3. The relation of free growth and of polymorphonuclear phagocytosis to resistance in tuberculosis is discussed.

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DESCRIPTION OF PLATES

PLATE 162

FIG. 1. One of the large clumps of tubercle bacilli which characterized inoculum employed in most of the experiments. Photomicrograph taken with blue light. × 1500.

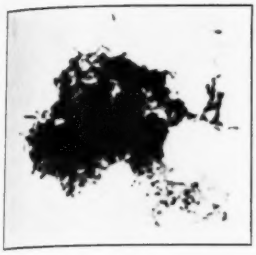
FIG. 2. Numerous polymorphonuclears in omental spread. Two hours after inoculation. Most of the cells have phagocytosed tubercle bacilli. Blue light. × 330.

FIG. 3. Polymorphonuclear cells with phagocytosed bacilli. From omental spread made 4 hours after inoculation. Blue light. × 1500.

FIG. 4. Large mononuclear cell showing phagocytosis of polymorphonuclears which had taken up bacilli. Found in smear of peritoneal fluid 24 hours after inoculation. Blue light. × 1500.

FIG. 5. Cell from same preparation as Fig. 4. Blue light. × 1500.

FIG. 6. Cell of mononuclear series which has phagocytosed bacilli directly. From omental spread made 48 hours after inoculation. Blue light. × 1800.



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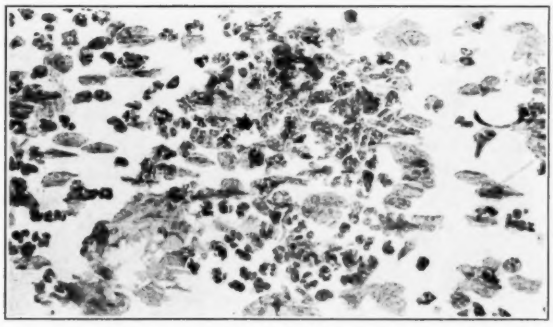

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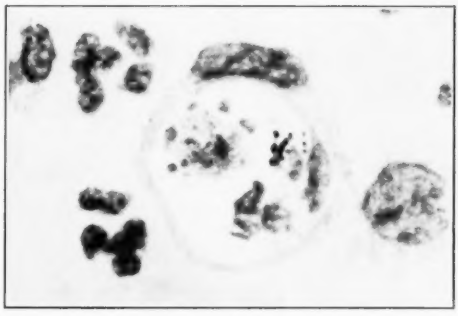

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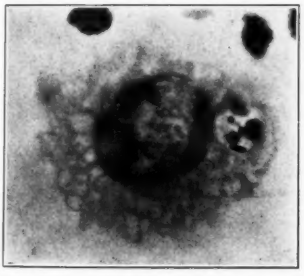

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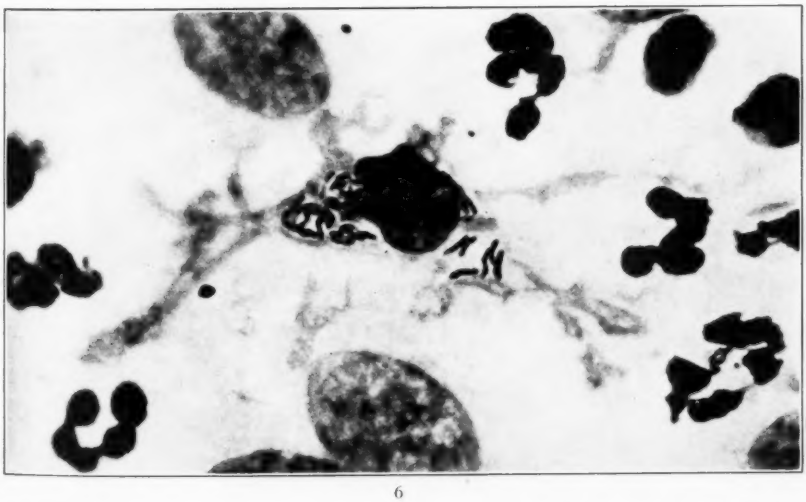

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PLATE 163

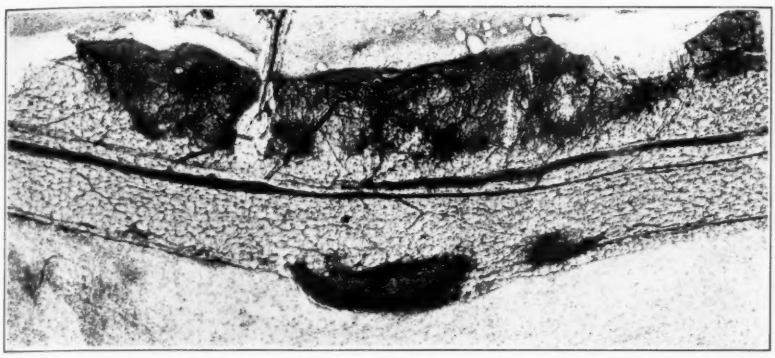
FIG. 7. The dark areas in the photograph are masses of polymorphonuclear leukocytes which have migrated to the perivascular fat tissue. From omental spread made 24 hours after inoculation. White light. × 25.

FIG. 8. Omental spread made 3 days after inoculation. Large mononuclear cells replace the polymorphonuclears and almost completely obliterate the perivascular fat cells. Arrow points to a circle of cells formed as the result of the localization of a bubble of air introduced at time of intraperitoneal inoculation. White light. × 25.

FIG. 9. Omental spread 4 days after inoculation showing clump of large mononuclear cells. These cells seem to proliferate locally, by amitotic division, to form miliary tubercles. Blue light. × 600.

FIG. 10. More advanced stage of miliary tubercle formation, with freely growing bacilli on cell outlined in rectangle. Omental spread made 4 days after inoculation. Blue light. × 165.

FIG. 11. Higher magnification of cell outlined in Fig. 10. Freely growing bacilli are clearly shown. Blue light. × 1500.



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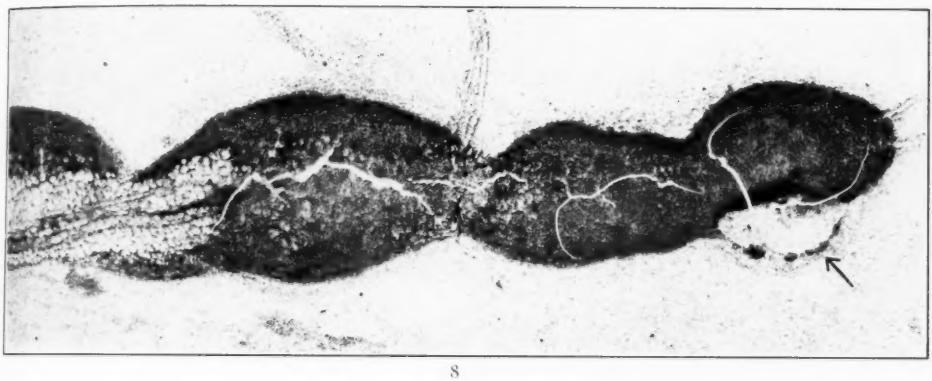

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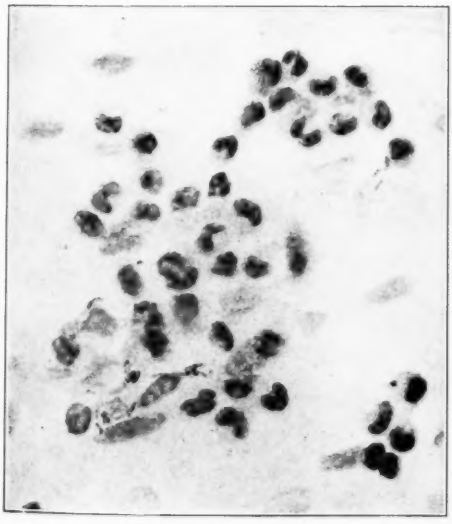

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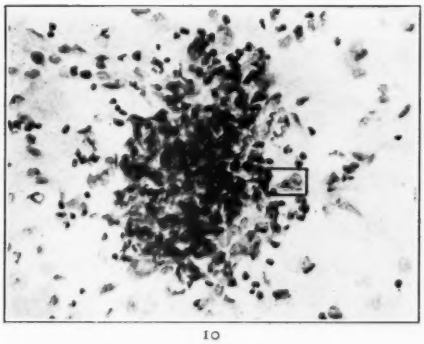

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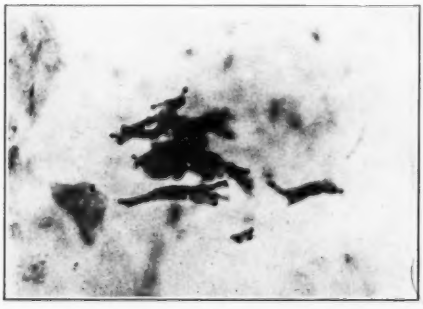

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Woodruff

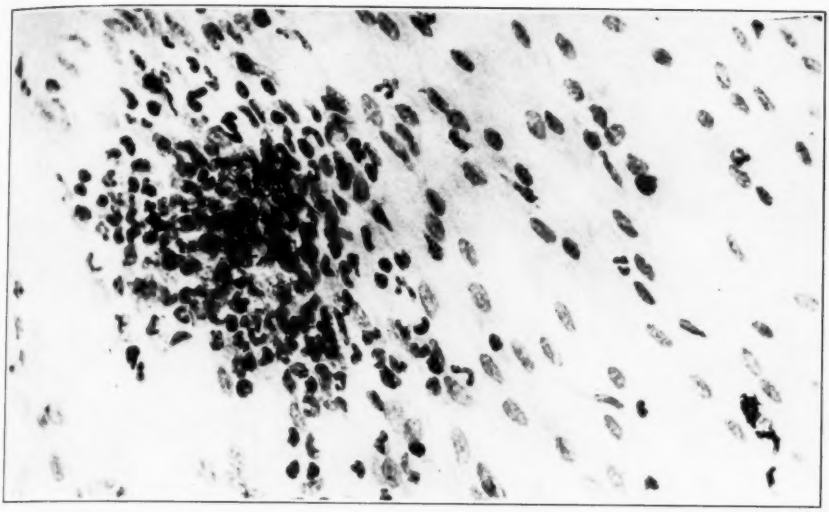
Free Growth Period of Tubercle Bacilli

PLATE 164

FIG. 12. Freely growing bacilli, in lower right hand corner, at some distance from a developing tubercle. Omental spread made 6 days after inoculation. Blue light. × 330.

FIG. 13. Same tubercle and bacilli as shown in Fig. 12. Photomicrograph taken with green light to bring out the elastic network which characterizes the guinea pig's omentum. Green light. × 300.

FIG. 14. Numerous colonies of freely growing bacilli on omental cells 6 days after inoculation. Blue light. × 600.



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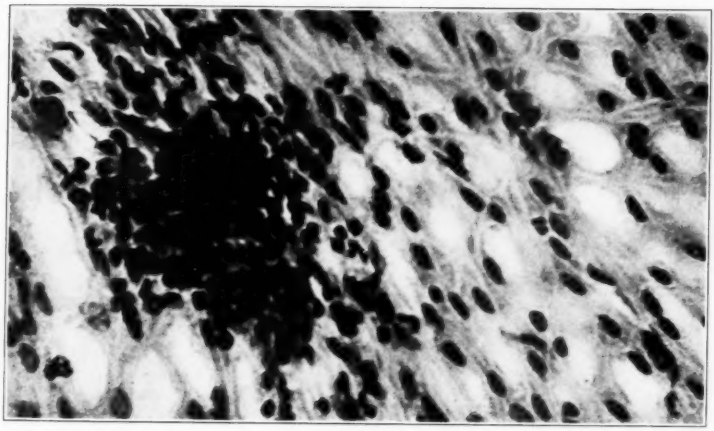

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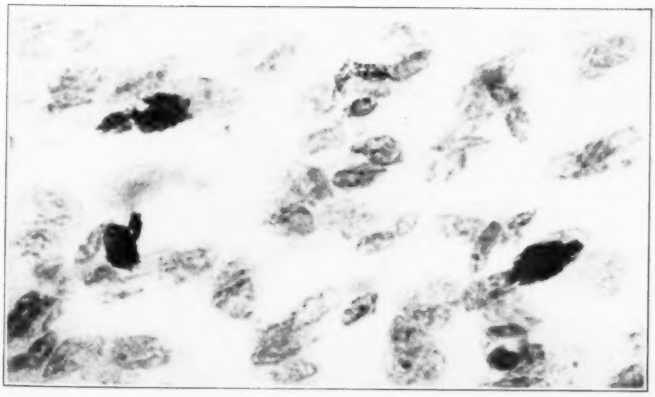

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Plate 165

Fig. 15. Freely growing bacilli on omental cells 6 days after inoculation. Blue light. × 1500.

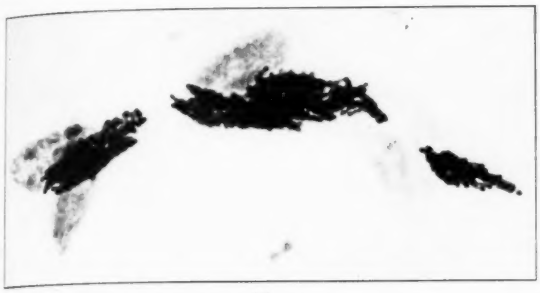
Fig. 16. Freely growing bacilli on omental cell 6 days after inoculation. Frequently the bacilli curve about the cell nucleus as shown. Blue light. × 1800.

Fig. 17. Freely growing bacilli on omental cells 6 days after inoculation. To all appearances the affected cells are no different from the surrounding cells. Blue light. × 350.

Fig. 18. Higher magnification of cells outlined in Fig. 17. Blue light. × 1800.

Fig. 19. Freely growing bacilli at some distance from a large tubercle formed 7 days after inoculation with caseous material. Tubercle formation was more rapid in this series than after inoculation of H-37 from culture. Blue light. × 165.

Fig. 20. Higher magnification of bacilli outlined in Fig. 19. Blue light. × 1500.



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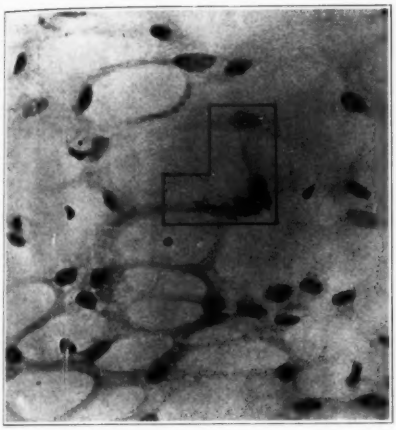

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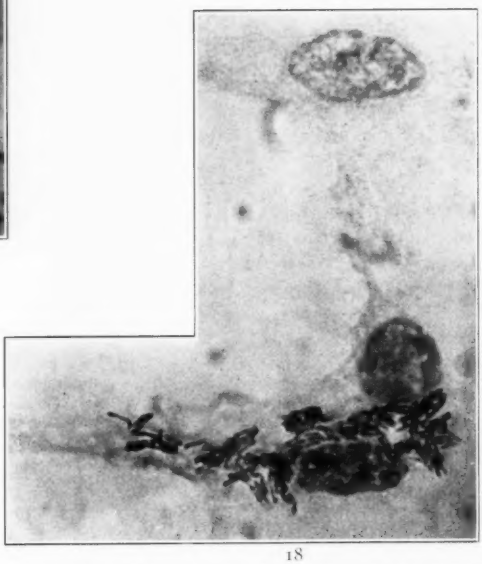

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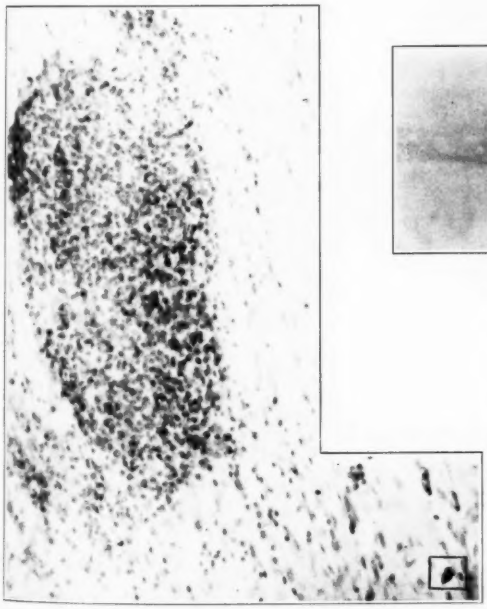

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Woodruff

Free Growth Period of Tubercle Bacilli

PLATE 166

FIG. 21. Tubercles in omental spread made 48 hours after inoculation with timothy grass bacilli. Tubercle formation in this series, too, proceeded more rapidly than after H-37 inoculation. White light. × 165.

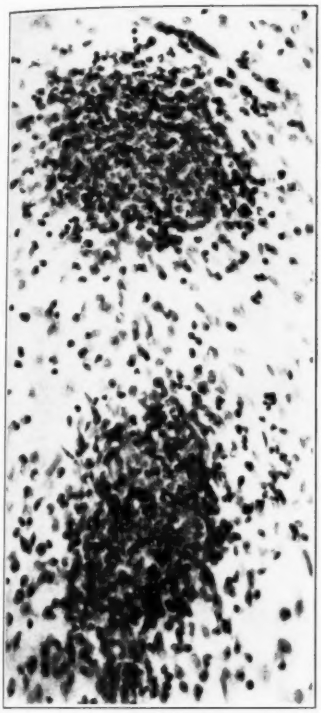
FIG. 22. Tubercle 6 days after inoculation with timothy grass bacilli. White light. × 165.

FIG. 23. Omentum of hypersensitive guinea pig 48 hours after inoculation. A clump of bacilli from the original inoculum still exists. Blue light. × 330.

FIG. 24. Smear of caseo-pus found encapsulated in omentum of hypersensitive guinea pig 10 days after inoculation. The smear showed numerous clumps of bacilli closely surrounded by polymorphonuclear cells. Blue light. × 330.

FIG. 25. Same clump of bacilli as shown in Fig. 24. Photomicrograph taken with white light to bring out the polymorphonuclears. White light. × 1500.

FIG. 26. Same bacilli as in Fig. 25. Photomicrograph taken with blue light. × 1500.



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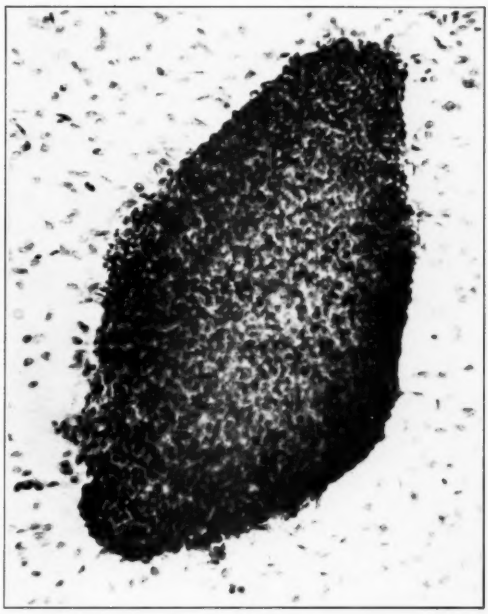

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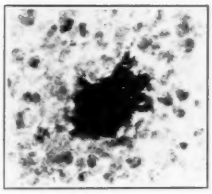

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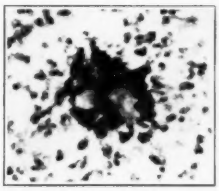

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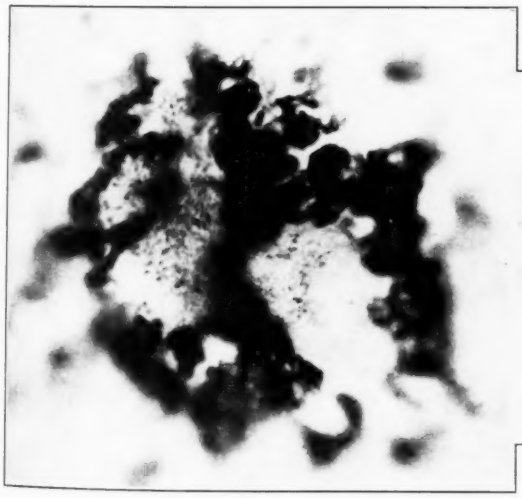

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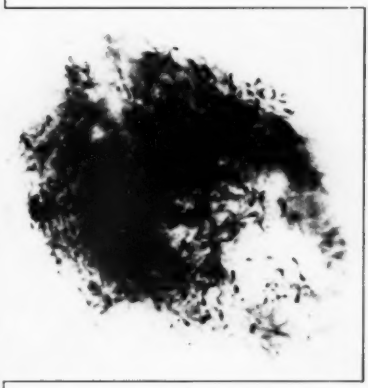

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Woodruff

Free Growth Period of Tubercle Bacilli

PLATE 167

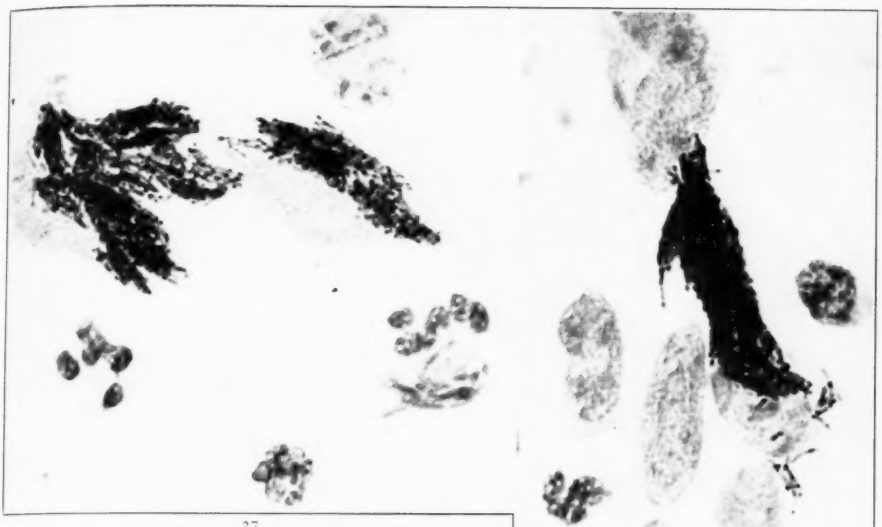
FIG. 27. Freely growing bacilli with adjacent polymorphonuclear cells. Omental spread made 7 days after inoculation. Blue light. × 1500.

FIG. 28. Freely growing bacilli with adjacent polymorphonuclear cells. Seven days after inoculation. Blue light. × 1500.

FIG. 29. Freely growing bacilli 7 days after inoculation. A polymorphonuclear leukocyte has applied itself to right hand edge of the colony. Blue light. × 1500.

FIG. 30. Colony of freely growing bacilli being invaded and broken up by polymorphonuclear leukocytes. Omental spread made 7 days after inoculation. Blue light. × 1500.

FIG. 31. Same group of bacilli as shown in Fig. 30. Photomicrograph taken with white light to bring out polymorphonuclears. White light. × 1500.



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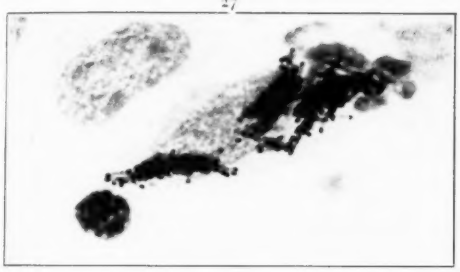

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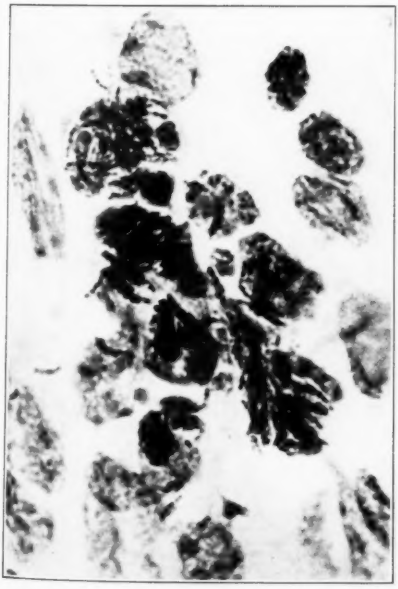

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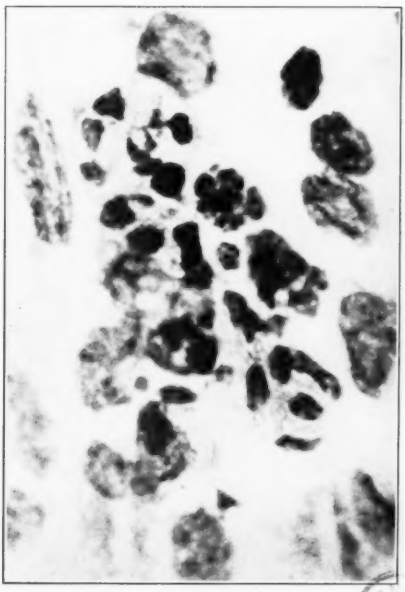

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Woodruff

Free Growth Period of Tubercle Bacilli

PLATE 168

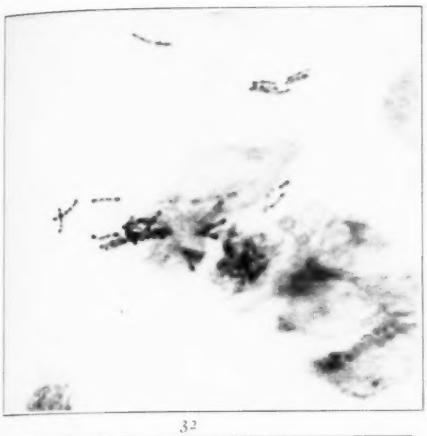
FIG. 32. Showing marked beading of the freely growing bacilli, 7 days after inoculation. Blue light. × 1500.

FIG. 33. Two "vintages" of tubercles commonly found in omentum 9 days after inoculation. White light. × 165.

FIG. 34. Higher magnification of small tubercle shown in upper corner of Fig. 33. Blue light. × 1500.

FIG. 35. Tubercle bacilli of bizarre shape. From omental spread made 11 days after inoculation. Blue light. × 1500.

FIG. 36. Bacilli in omental spread made 37 days after inoculation. Blue light. × 1800.



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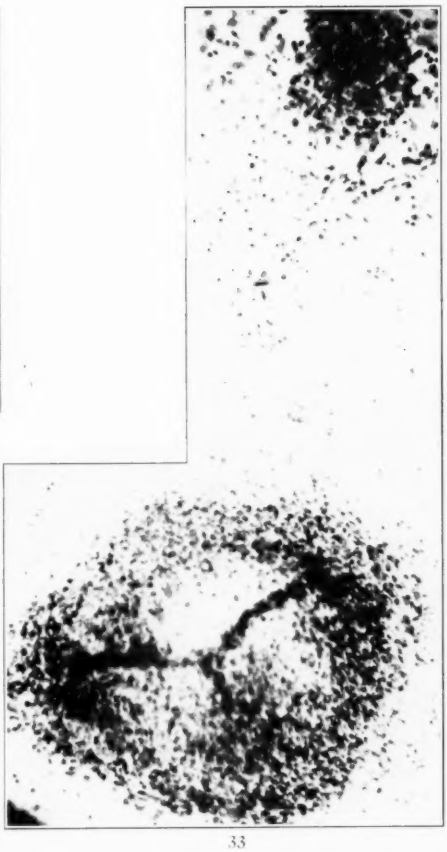

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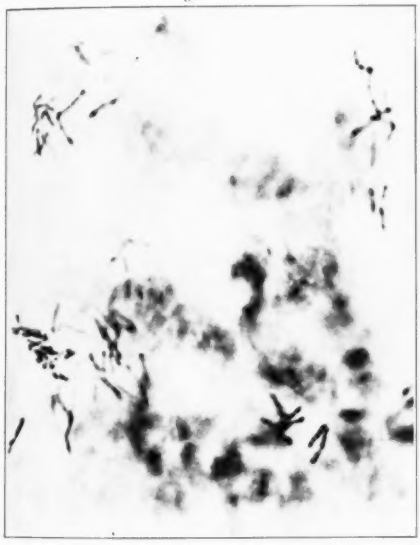

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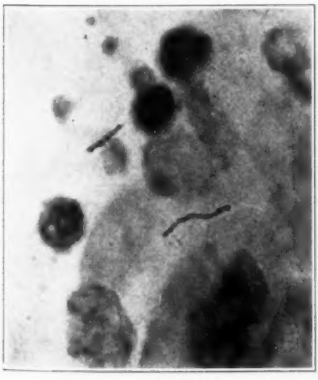

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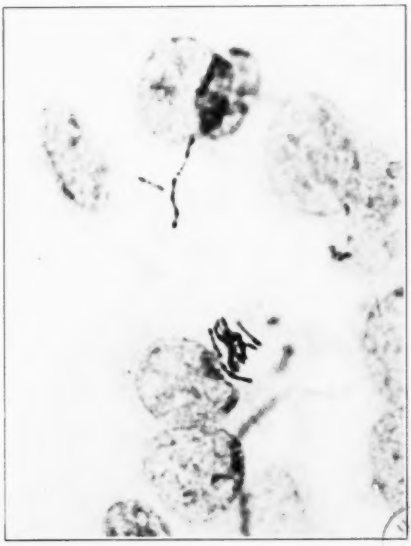

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Woodruff

Free Growth Period of Tubercle Bacilli

SYPHILITIC ANEURYSM OF LEFT CORONARY ARTERY WITH CONCURRENT ANEURYSM OF A SINUS OF VALSALVA, AND AN ADDITIONAL CASE OF VALSALVA ANEURYSM ALONE *

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CORONARY ARTERY ANEURYSMS

The rarity of such aneurysms, exclusive of the false or dissecting forms and those associated with periarteritis nodosa, is stressed by Karsner [1] and further attested by the few reports in the literature. Packard and Wechsler,[2] 1929, in their classical survey, could find only 30 examples, the first of which was recorded by Bougon [3] in 1812. Packard and Wechsler have thoroughly reviewed all reports of this condition, checked duplications and culled out questionable examples, so that we need only to cover the literature since 1929 and summarize briefly the salient facts elicited by these authors. They placed coronary aneurysms in two etiological groups: (1) mycotic-embolic, associated with bacterial endocarditis involving the aortic valve; and (2) arteriosclerotic, related to coronary sclerosis and long continued hypertension. The average age of the first group was 27 years, as compared with 57 years for the latter. There is no mention of syphilitic coronary arteritis as a cause of aneurysm of these vessels and mesaortitis was present in only 3 of the 30 collected cases. Rarely, indirect trauma due to strain may have been an etiological factor, but direct injury had an insignificant rôle, apparently on account of the protected anatomical site of the coronary vessels. In nearly all cases the aneurysms were single and located within the first inch of the left coronary artery, which was involved about three times as frequently as the right. In 4 hearts both coronaries were attacked. About 50 per cent of the aneurysms ruptured. There were no pathognomonic symptoms or signs that permitted an ante mortem diagnosis of the condition.

Cox and Christie,[4] 1930, described a fusiform aneurysm 2.5 by 4.5 cm. involving the right coronary artery. This was associated

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with cardiac hypertrophy (500 gm.), old and organized thrombosis of the anterior interventricular branch of the left coronary artery, myocardial fibrosis and aneurysm of the abdominal aorta and of the right common iliac artery. Microscopic study of the aorta revealed only marked arteriosclerosis. Apparently no histological study was made of the coronary aneurysm. The lesion occurred in a white male 65 years old with a long history of vascular hypertension and of a paralytic "stroke."

Vogelsang,[5] 1930, reported an aneurysm 6 by 5.5 by 4.5 cm. involving the anterior interventricular branch of the left coronary artery and situated in the anterior wall of the left ventricle. There were also gummatous myocarditis, syphilitic aortitis and gummatous pneumonitis. Distal to the aneurysm the artery was thrombosed. He included no microscopic description of the aneurysm, but expressed the opinion that in view of the gross and microscopic lesions elsewhere the aneurysm was probably also of syphilitic origin, and that the trauma of a 9 foot fall which the man, a 38 year old seafarer, sustained 4 months prior to his sudden death, might have been an etiological factor.

Halpert,[6] 1930, described an arteriovenous communication between the right coronary artery and the coronary sinus, with aneurysmal dilatation of both, in a man 54 years of age who showed no cardiac disturbances during life and died of gastric carcinoma. From gross and microscopic investigation he concluded that the lesion probably was congenital.

Thus, to date (May 1934), 33 acceptable cases of coronary aneurysm have been recorded, and to this total we desire to add another, the only one of its kind among 5896 autopsies performed by the pathology department of the University of Oregon Medical School.

Aneurysm of the Aortic Sinuses of Valsalva

This is another uncommon site of aneurysm formation, but is less rare than in the coronary arteries. The comparative rarity of such lesions, together with the complications peculiar to their anatomical location, warrant the recording of further instances. In our autopsy material 2 cases have been encountered, one complicated by aneurysm of the left coronary artery, the other occurring in conjunction with syphilitic aortitis.

Valsalva aneurysms of congenital,[7] syphilitic,[8, 9, 10] arteriosclerotic,[9] mycotic,[9, 11] and indeterminate [12] etiology have been described as have dissecting or false aneurysms. Often these rupture, usually into the pericardial sac, the chambers of the heart or into the great vessels, but may burst externally after eroding the chest wall, as in the case of Sheldon,[13] in which the pericardial sac had been obliterated by fibrous adhesions, presumably incident to rheumatic disease. Without rupturing, these aneurysms may burrow into the myocardium of the atria, ventricles or interventricular septum and in this way produce stenosis or insufficiency of one or more valves. Another complication, present in one of our cases, is heart block from encroachment upon the atrioventricular bundle, while still another, exemplified by our Case 1, is extension of the aortic disease to an adjacent coronary artery with the formation of an aneurysm in this vessel as well.

Most frequently the anterior sinus is involved, due, according to von Krzywicki,[9] to its unsupported position superior and somewhat anterior to the membranous interventricular septum. Gray [14] considers that the regurgitation of blood, directed chiefly against the anterior aortic wall, is a factor also.

The actual incidence of Valsalva sinus aneurysms is difficult to ascertain since most authors who have made statistical studies have not only failed to distinguish between true and dissecting aneurysms, but in addition have not made clear as to whether or not their figures are based only upon adequately described and complete autopsies. In many compilations the location of the aortic aneurysms is given only within wide anatomical limits. It is probable that some Valsalva aneurysms have been included with those of the ascending aorta and it is possible that others are buried in reports with misleading titles.

In order to obtain figures of incidence of various aortic aneurysms in a series of autopsies we have reviewed all of our records and have disregarded 605 protocols because the examinations were incomplete, or the description of the aorta was unsatisfactory. Among these were 13 cases in which one or more sacculations were present. Among the remaining 5896 autopsies 143 individuals had 214 true aneurysms of the aorta distributed anatomically as follows:

	No.	Per cent
Sinuses of Valsalva	2	0.93
Ascending aorta	102	47.66
Ascending aorta and transverse arch	11	5.14
Transverse arch	35	16.35
Arch and thoracic	1	0.47
Ascending, arch and thoracic	3	1.40
Thoracic	43	20.10
Abdominal	17	7.94

In 20 persons there were 26 dissecting aneurysms which are not included in the tabulation. Among those listed were 6 cases of concomitant true and dissecting aneurysm, but the latter have been omitted from the compilation. In the tabulated group the probable etiology was: syphilitic 87.8 per cent, arteriosclerotic 10.3 per cent, mycotic-embolic 1.4 per cent and rheumatic 0.47 per cent. These figures agree fairly well with those of Brindley and Schwab,[15] but in our series syphilis seems to be a somewhat more prominent etiological factor, possibly because only true aneurysms are included.

We have made no exhaustive survey of the literature dealing with aneurysms of the sinuses of Valsalva but wish to call attention to the statistics of Smith[10] and of Lucké and Rea[16] who found 10 cases among 287 aortic aneurysms in 12,000 autopsies collected from various sources.

Case Reports

Case 1. *Syphilitic Aneurysm of the Left Coronary Artery with Concurrent Aneurysm of Sinus of Valsalva:* The clinical history and autopsy record were not available.

Postmortem Examination

The heart and 6 cm. of the ascending aorta had an aggregate weight of 595 gm. The intima of the ascending aorta was wrinkled, roughened, pearly gray to whitish and mottled by irregular yellowish areas. The wall was irregularly thickened, of cartilaginous consistence and in a state of saccular aneurysmal dilatation. The epicardium and endocardium were grossly unchanged. The left ventricle was greatly hypertrophied and dilated. The trabeculae carneae and papillary muscles were much enlarged, elongated and flattened. The mitral leaflets were unchanged. The aortic leaflets felt gristly and had thickened, rolled, rounded edges. The aortic ring had a circumference of 8.5 cm. The left atrial wall was 3 to 4

mm. thick across the pectinate muscles, but between them was almost transparent. There were two right coronary ostia, situated 2 mm. apart. The smaller was less than 1 mm. in diameter and led to a vessel coursing over the conus arteriosus. The orifice of the main artery was slit-like and measured 1 by 3 mm. Both ostia opened 1.1 cm. superior to the upper border of the anterior aortic leaflet. Serial cross-sections of the artery revealed some eccentric thickening of its wall by atherosclerotic plaques which did not close the lumen.

In the left posterior aortic sinus was an aneurysm having a crescentic opening measuring 0.7 by 1.8 cm. Within the anterior wall of the left ventricle this aneurysm expanded, attained dimensions of 3.1 by 3.4 by 2.4 cm. and became filled by a laminated thrombus. Anteriorly and to the right the sac bulged into the right ventricle directly below the posterior leaflet of the pulmonic valve, elevating it somewhat, and producing a triangular stenosis of the valve. The base of the triangle was formed by the bulging aneurysm wall and the opening of the valve was reduced to about half its normal size. The lining of the aneurysm resembled that of the aorta. The inferior wall of the sac was formed by the left ventricular myocardium and did not encroach upon the membranous part of the interventricular septum, but lay somewhat anterior to it. The sac also bulged into the left atrium beneath the anterior leaflet of the mitral valve, elevating it to some extent and producing a slight degree of stenosis. The right ventricle was markedly dilated and its wall was considerably hypertrophied.

The left coronary artery and the Valsalva aneurysm were examined by gross serial cuts, as shown in Figure 3. The left coronary ostium (Fig. 3A) lay 0.7 cm. above the tip of the left posterior aortic leaflet, was oval, measured 3 by 5 mm. and had its long diameter in the superior-inferior direction. It was surrounded by pearly white, wrinkled and greatly thickened aortic intima. Within 5 mm. of its origin (Fig. 3B) the main trunk enlarged to an outside diameter of 2.1 by 0.9 cm. Adherent to the rigid intima was a film of dry, blackish blood clot and even at this point the inferior half of the lumen was occluded by a laminated brownish gray thrombus. The vessel wall was from 1 to 3 mm. thick and fused with the aortic wall. Six mm. distal to the point just described (Fig. 3C) a marked change occurred. The coronary artery was now 2.3 cm. in one diameter and 2 to 4 mm. in the other. The wall had a thickness of 1 to 2 mm. and

over the superior part the lining was coated with blackish blood. Inferiorly the vessel came to a sharp point and here the thrombus observed in the previous block plugged the lumen. In the middle of this segment was the ostium of the circumflex branch of the left coronary artery, also occluded by the thrombus. It must be understood, then, that the dimensions given above represent not only the main coronary artery but also the beginning of its circumflex branch, and because this was cut tangentially the lower part of its lumen appeared pointed. On the right side of the coronary artery lay the Valsalva aneurysm containing a laminated thrombus, most of which dropped out in the process of sectioning. The coronary artery and the Valsalva aneurysm were separated by a hard and fibrous wall only 2 mm. thick. Four mm. distally (Fig. 3D), measuring along the coronary artery, was the beginning of the anterior interventricular branch. The main left vessel was still rather larger than normal, with an external diameter of 1.1 by 0.7 cm., but had decreased appreciably from its size in the preceding block. Its lumen had an undulating outline and the intima was covered by a thin, blackish film. The common wall dividing the artery and the Valsalva aneurysm appeared wholly fibrous and was 2 to 3 mm. thick. At this point the Valsalva sac measured 2.3 cm. in its transverse diameter, 3.4 cm. in the supero-inferior direction and had a wavy and whitish border. In the myocardium of the left ventricle forming the lower border of the aneurysm were many engorged capillaries within a whitish scar. In this block the circumflex artery had fully emerged as an independent vessel and exhibited nothing abnormal. The thrombus occluding the beginning of this vessel did not continue further along its course. The myocardium under the coronary aneurysm was only 6 mm. thick. The anterior interventricular branch, 5 mm. distal to the point previously described (Fig. 3E), was shaped like a bowling pin, with maximum external diameters of 1.2 and 0.6 cm. Its lumen was entirely closed by a dry, blackish clot. The same common wall, still 2 mm. in thickness, separated the vessel and the Valsalva aneurysm, which now measured 3.5 by 2.5 cm. The latter had become more superficial and was underlaid by 1.1 cm. of myocardium. The myocardial scar mentioned above continued into this block. The outside dimensions of the coronary artery were now 7 and 9 mm. In the next block (Fig. 3F), having a thickness of 5 mm., the Valsalva aneurysm had practically left the myocardium

and had come to lie chiefly in the subepicardial fat. Its dimensions were decreasing, being 3 by 2 cm. The wall of the sac and the thrombus contained within it were identical with previously mentioned blocks. The myocardial scar, however, had practically disappeared. The coronary artery and the Valsalva aneurysm continued to share a common wall and the thrombus occluding the lumen of the artery in more proximal blocks was still present. The external diameters of the vessel were the same as in the preceding block although the contour was different. Within the next 5 mm. (Fig. 3G) the Valsalva aneurysm had fully emerged from the myocardium, lay wholly in the subepicardial fat and decreased in size to 2.3 by 1.8 cm. The walls of the anterior interventricular artery and the aortic sinus aneurysm were still in apposition but no longer fused into one. The thrombus mentioned previously continued and the coronary artery measured 5 by 7 mm. Three mm. distally (Fig. 3H), the Valsalva aneurysm terminated blindly in the subepicardial fat, 4 mm. beneath the epicardium, and had not ruptured. The relations of the two aneurysms are shown clearly in the wax reconstruction (Fig. 2).

Microscopic Examination

Microscopically the clots filling the Valsalva sacculation and the aneurysm of the left coronary artery prove to be typical laminated thrombi exhibiting some softening but no organization.

The wall of the Valsalva aneurysm consists almost entirely of hyalinized fibrous connective tissue in which the Van Gieson and Verhoeff stains display persisting remnants of both smooth muscle cells and elastic tissue. Numerous partially or completely obliterated vasa vasorum are collared by abundant plasma cells and lymphocytes. The medial and intimal divisions are not clearly distinguishable on account of the great distortion and fibrosis, while in the inner part of the wall there are extensive atherosclerotic changes and calcification. The common wall separating the Valsalva and coronary artery aneurysms is made up of hyalinized fibrous tissue and granulation tissue containing fragments of elastic and smooth muscle tissue. Here also are small vessels showing obliterative changes and perivascular cuffs of lymphocytes and plasma cells. It appears obvious that this common wall represents a fusion of the walls of the aneurysmal aortic sinus and the left coronary artery.

The left coronary artery wall exhibits changes identical with those just described for the Valsalva aneurysm and these are depicted in Figure 4. The myocardium subjacent to the aortic sinus aneurysm gives histological evidence of pressure atrophy, hyaline degeneration, interstitial fibrosis and contains numerous small and engorged blood vessels. Other sections of the myocardium reveal a distinct hypertrophy of the muscle cells with deposits of lipochrome pigment at either end of the nuclei, slight patchy interstitial fibrous connective tissue increase and multiple small areas of anemic necrosis in blocks coming from the anterior wall of the left ventricle. The intima of the ascending aorta is the seat of typical atherosclerosis with calcification, while the media displays extensive destruction of smooth muscle and elastic tissue. The vasa vasorum of both media and adventitia are greatly narrowed or obliterated by endothelial proliferation and intimal fibrosis and are collared by plasma cells and lymphocytes. Some of these accumulations show early necrosis and are regarded as actual miliary gummas.

CASE 2. *Syphilitic Aneurysm of Aortic Valsalva Sinus:* The patient, a white male, 60 years old, spoke and understood so little English that an adequate history was unobtainable. He had been working "in the woods," presumably at logging, until about the first of November, 1932, when he became short of breath, progressing to orthopnea within 2 months. About the first of January, 1933, there developed a continuous, dull, distressing pain in the epigastrium and right upper abdomen. Swelling of the ankles appeared about a month later. He was weak, "nervous," could not sleep, and had been confined to bed most of the time since the appearance of the edema. Occasionally he had noted substernal distress and tinnitus. He was admitted, walking, to Multnomah County Hospital on Feb. 21, 1933.

On admission the temperature was 95 F, pulse 88, respiration 24 and the blood pressure 160/35. Physical examination revealed slight pallor and cyanosis of the finger nails, but no demonstrable capillary pulse. The retinal blood vessels pulsated and the optic discs appeared hazy. The neck vessels pulsated markedly with systole. The chest was slightly emphysematous. The cardiac impulse was diffuse and heaving. There was slight impairment to percussion between the right scapula and the spine, with bronchovesicular breathing and a to-and-fro murmur. Elsewhere the lung fields seemed normal. To percussion the heart was of the "aortic" configuration, the arch 6 cm. wide, and the left and right borders respectively 14 and 6 cm. from the midsternal line. A loud to-and-fro murmur was audible over all valve areas, loudest at the aortic and mitral areas and transmitted to the axilla and through to the back. No distinct valve tones could be heard. The peripheral vessels were thick-walled and pulsated forcibly. The abdomen was distended. The liver border was 6 cm. below the costal margin and was felt also in the epigastrium. The legs were very edematous.

The urine was negative. Except for a sedimentation rate of 13 mm. in 15 minutes and 37 mm. at the end of 45 minutes (modified Westergren method), and 4 plus Kolmer and Kahn reactions, examination of the blood yielded results within normal limits.

An electrocardiogram taken on the day of admission showed the auricular and ventricular rates to be each 71. T_1 was inverted, R_1 slurred, Q-S prolonged and the P-R interval prolonged to 0.27 second. T_2 was inverted, R_2 slurred, and P-R prolonged. R_3 was slurred and notched, P_3 and T_3 were questionably diphasic and R_3 of low amplitude. The interpretation was: "Delayed A-V conduction, coronary type T-waves, myocardial damage."

Under a regimen of bed rest, digitalis, sedatives, bismuth and iodides, the patient improved and 3 days later the peripheral edema was gone. On February 27th a roentgenogram of the chest showed increased hilum shadows, pleural thickening on the right, with obliteration of the costophrenic angle, and a greatly enlarged cardiac shadow with a blunt apex and a somewhat widened and sclerotic arch.

By March 2nd there was no dyspnea or cyanosis. The man's condition remained unchanged until April 15th, when he had diarrhea and abdominal distress. Six days later he suddenly became cyanotic and died.

The final clinical diagnoses were: (1) Aortic regurgitation on the basis of syphilitic destruction of the aortic ring; syphilitic aortitis; hypertrophy and dilatation of the right and left heart with cardiac failure, functional capacity II–B, and peripheral edema. (2) Mild chronic hypertrophic emphysema.

Postmortem Examination

No. 163–4–33. Examination of the abdomen revealed slight ascites, a non-specific diphtheritic, hemorrhagic and ulcerative enterocolitis and proctitis, and chronic passive hyperemia of the liver with acute periportal hepatitis, and chronic passive congestion of the spleen and kidneys.

The left pleural cavity contained about 3 liters of clear fluid, which compressed the lung inferiorly and posteriorly. The right lung was bound to the chest wall by dense adhesions at its apex, and laterally and inferiorly over its middle and lower lobes. Some of these adhesions, which were fibrous in nature, obliterated the right costophrenic sinus and encapsulated some yellowish white, cheesy and hyaline material. The space occupied by the heart was greatly increased, having a maximum transverse diameter of 19 cm. All cardiac chambers were much dilated and more than half of the anterior presenting surface was formed by the right ventricle and atrium. The papillary muscles and trabeculae carneae of both ventricles were appreciably elongated, thickened and also flattened.

With the heart opened there could be seen immediately inferior to the pulmonic valve a rounded, bulging area forming a sort of

shelf on the septal wall of the right ventricle and producing some stenosis immediately proximal to the valve. Over the inferior half of this projection the endocardium was pearly white and the superior border of the whitened area was quite sharp, due to the fact that at this point the interventricular septum had been reduced to a hyaline state and formed one wall, here only 2 mm. thick, for the Valsalva sinus aneurysm to be described in more detail presently. Several of the chordae tendineae of the anterior cusp of the tricuspid valve were attached to the aneurysm wall which lay immediately anterior and to the left of this valve. The aortic valve measured 9 cm. in circumference and its leaflets were somewhat rigid, with thickened and rolled margins. The commissures between the leaflets were widened, the distance between the right and left posterior leaflets being 1 mm., while the commissures separating the anterior and left posterior leaflets and the anterior and right posterior leaflets were each 3 mm. At a point 4 cm. superior to the aortic ring the aorta had a circumference of 12.5 cm. The ostium of the right coronary artery was slit-like, measured 1 by 2 mm. and was situated 6 mm. superior to the tip of the anterior aortic leaflet on the superior aspect of the shelf-like margin of a semilunar aneurysm 2 by 1.3 cm. in size, which occupied the right posterior sinus of Valsalva and extended into the interventricular septum for a distance of 2.8 cm. (Fig. 5). In its development the aneurysm left behind a narrow and firm ridge separating it from the attachment of the right posterior aortic leaflet. The lining of the sac was rough, grayish white, mottled by yellowish atheromatous plaques, of cartilaginous firmness and displayed over a part of its blind end a thin, grayish red thrombus. The wall forming the blind end lay in a concavity hollowed out of the muscular interventricular septum. The pars membranacea septi appeared to have been displaced to the left to form most of the left wall of the aneurysm, which, however, did not stop here but continued downward, cupping out for itself a bed in the muscular interventricular septum. Along the right border of the shelf bearing the right coronary orifice was a deep and narrow vertical groove communicating directly with the Valsalva aneurysm. The ostium of the left coronary artery measured 2 by 3 mm. and opened 2 cm. superior to the upper boundary of the left posterior aortic leaflet. Serial cross-sectioning of the coronary arteries revealed them to be macroscopically unchanged. The wall of the en-

tire aorta was irregularly thickened and distorted and the vessel was quite tortuous. The intima, from the root to the bifurcation, was whitened, thickened and wrinkled longitudinally. Toward the bifurcation was an increasing amount of atheromatous change with ulceration and calcification. The vessel cut with leathery resistance and was unduly adherent to the structures about it. In the anterior wall of the abdominal aorta was a small saccular outpouching. The heart and entire aorta weighed 940 gm. The myocardium of both ventricles was distinctly hypertrophied and pinkish, with a slight yellowish mottling. The anterior wall of the left ventricle seemed to be elongated. Fibrotic patches were noted at the tips of the various papillary muscles. The mitral valve ring was 11.5 cm. in circumference and its leaflets, particularly the anterior one, were flecked by atheromatous patches.

In the lower lobe of either lung were several rubbery, yellowish to grayish, sharply circumscribed, grouped nodules with polycyclic outlines. The bronchial and pulmonary arterial walls were thickened and some of the latter displayed atheromatous plaques.

Microscopic Examination

Microsections of the septal portion of the Valsalva aneurysm reveal a partially organized and partly softened thrombus at its base. The wall of the sac is formed by a thick layer of hyaline material showing atheromatous changes and hemosiderin deposits. Blending with this is a layer of vascular granulation and fibrous scar tissue containing numerous partially or completely obliterated arteriolar channels surrounded by broad collars of lymphocytes and plasma cells. By means of the Verhoeff stain fragments of degenerating elastic tissue are identified, proving that this portion of the sac represents remnants of aortic media. Separating this layer and the septal myocardium is a thin zone of connective tissue, probably representing aortic adventitia, containing a number of tiny vascular channels, apparently veins. Farther out are degenerated cardiac muscle cells, isolated or split up into small groups and compressed by dense fibrous tissue. On account of the formalin fixation and the time elapsing between death and autopsy, special stains to demonstrate the cells of the atrioventricular bundle were not employed.

Sections of the aorta, taken at various levels from the root to the bifurcation, all exhibit essentially the same changes, namely, irregular thickening, fibrosis, puckering and distortion of all layers, with extensive fragmentation and destruction of medial smooth muscle and elastic tissue with partial replacement of these by fibrous connective tissue of varying age. The aortic vasa vasorum display all degrees of obliterative endarteritis and are surrounded by varyingly dense collars of lymphocytes, plasma cells, Russel-Plimmer bodies and occasional polymorphonuclear eosinophiles. Certain of the cellular accumulations show slight necrosis. The adventitia is the seat of similar changes.

Sections of myocardium from either ventricle exhibit marked hypertrophy and deposition of pigment at the nuclear poles with distinct cross-striations in some cells and an absence of these in others. A few of the cells appear to be atrophic. The interstitial tissue seems to be slightly edematous, a little increased in places and contains occasional lymphocytes and plasma cells but no cellular aggregations resembling gummas. The smaller branches of the coronary arteries are unchanged. Frozen sections stained with scharlach R fail to show any fat in the myocardium.

Microscopic examination of the lower lobes of the lungs reveals compression atelectasis, chronic passive hyperemia, edema, slight emphysema, and numerous pneumoliths. Here and there, in relation to bronchi or larger blood vessels are nodules composed of plasma cells and lymphocytes, surrounded by vascular granulation tissue containing many of these cells. Large areas of incomplete caseation necrosis in which the architecture of lung tissue and blood vessels can still be recognized are present. The necrotic tissue stains pinkish with eosin and there is no evidence of calcification or persistence of nuclear fragments, nor are any giant cells or epithelioid cells seen. No structures resembling daughter tubercles are in evidence. At the edges of such lesions the arterioles have undergone extensive obliterative inflammation. The larger branches of the pulmonary arteries are atheromatous and proliferative intimal changes have reduced their lumens to some extent. In places their walls contain a moderate number of lymphocytes, plasma cells, and rarely eosinophilic and neutrophilic polymorphonuclears. The right costophrenic pleural thickening consists of hyalinized fibrous

tissue and granulation tissue containing patchy accumulations of lymphocytes and plasma cells, forming a capsule about amorphous and hyaline material in which there is much cholesterin.

Discussion

In addition to the previously recognized causes for the development of aneurysm of the coronary arteries, such as mycotic-embolic infection and arteriosclerosis, we offer another, namely, syphilitic arteritis. In so doing we are cognizant of the truth of the generally accepted belief that the coronary arteries are only rarely affected by syphilis distal to their intra-aortic segments. However, in Case 1 there exists a most unique pathological condition which modifies the usual circumstances to such an extent that we have no hesitancy in terming the coronary lesion syphilitic. Undoubtedly the involvement of the left coronary artery was dependent first upon the localization of an active syphilitic aortitis in the left posterior sinus of Valsalva, and secondly the direction of burrowing of the enlarging sac which finally brought it into intimate contact with the main left coronary artery. There must then have been a spread of the *Spirocheta pallida* from the wall of the Valsalva sac to the wall of the coronary artery, with resultant destruction, fusion of the walls of the two juxtaposed structures and, finally, the formation of a true aneurysm in the weakened coronary artery.

As evidence of the syphilitic nature of the lesion we submit the microscopic observation of obliterative endarteritis of the vasa vasorum, perivascular collars of plasma cells and lymphocytes, microscopic sized gummas, destruction and scarring of the media and adventitial fibrosis, all of which are recognized as characteristic of syphilis by Warthin [17] and Moritz.[18] A careful study of the aorta, coronary arteries and myocardium failed to disclose anything that could be interpreted as rheumatic disease, a possibility which, in view of the newer histopathology of rheumatism, as described by Klinge and Vaubel,[19] and others, must be kept constantly in mind in the study of vascular lesions. It should be mentioned also that the vascular changes in the coronary artery of Case 1 were quite different from the commonly observed adventitial cellular infiltration accompanying coronary arteriosclerosis.

Another feature of this case makes it doubly interesting, for in addition to aneurysm of the coronary artery there was recent thrombotic occlusion of the sacculation and the lumen of the vessel adjacent to it. Closure of a main coronary artery near the heart would lead to serious consequences, even in a healthy organ, and in this instance would be even more embarrassing on account of the previously existent and plainly evident aortic insufficiency and dilatation of the left ventricle. Although the record and identifying number of the specimen are missing there is every reason for believing that coronary thrombosis was the terminal event of this person's life. Death evidently supervened shortly after the thrombus formed, for only the earliest indications of infarction of the myocardium were present.

While Vogelsang's [5] example of coronary aneurysm may well have been due to syphilis, one cannot be certain that such was the case because the vessel was not studied histologically. In our case, although spirochete stains were not done, it is felt that the evidence of syphilis is indisputable. With the possible exception of Vogelsang's case the present example appears to be the first of its kind thus far recorded.

In Case 2 it is possible that the incipient heart block, not evident clinically but suggested by the prolonged atrioventricular conduction time and the inverted T-waves of the electrocardiogram, may have been caused by digitalis, but we feel that the Valsalva aneurysm, on account of its size and anatomical relation to the atrioventricular bundle, together with degenerative and fibrotic changes in the adjacent myocardium, afford a more plausible explanation for these phenomena. Another factor contributing to cardiac failure was aortic insufficiency, which not only threw additional strain on the left ventricle but also failed to allow sufficient blood to enter the stenosed coronary ostia to nourish the myocardium properly. The right cardiac hypertrophy probably was due to increased pressure in the lesser circulation bed, due both to the stenosis of the pulmonary valve region by the Valsalva sinus aneurysm and also to pulmonary atherosclerosis which, in turn, probably followed increased circulation pressure from left heart failure on the basis of aortic insufficiency and stenosis of the mitral area from the Valsalva aneurysm. The presence of plasma cells and lymphocytes in the walls of the pulmonary arteries suggests that the sclerosis of these vessels may, in part at least, have been the result of syphilis.

The lesions in the bases of the lungs exhibited the characteristics of gummas and bore scarcely any resemblance to tubercles. Although the spirochete stains failed to demonstrate the organism it is our opinion, from the histological structure of the lesions and the known syphilitic nature of the aortic disease, that the pulmonary foci are very probably luetic as well.

The aortic involvement was much more extensive than is usual in syphilis and it is interesting to note that a second saccular aneurysm had developed in the abdominal division of the vessel.

Summary

Two cases of syphilitic aneurysm of the aortic sinuses of Valsalva with unusual complications are described. Such sacculations are distinctly uncommon, forming only 0.93 per cent of the aortic aneurysms in our series of 5896 autopsies.

In 1 case the aneurysm burrowed through the ventricular myocardium until it reached the left coronary artery, where a secondary syphilitic arteritis was established, leading first to aneurysm of the coronary artery and finally to acute thrombotic occlusion. This is the 34th case of coronary aneurysm to be recorded and apparently the first to have a syphilitic etiology.

The uncommon manifestation of Valsalva aneurysm in the second case was incipient heart block, dependent upon the proximity of the sac to the atrioventricular bundle.

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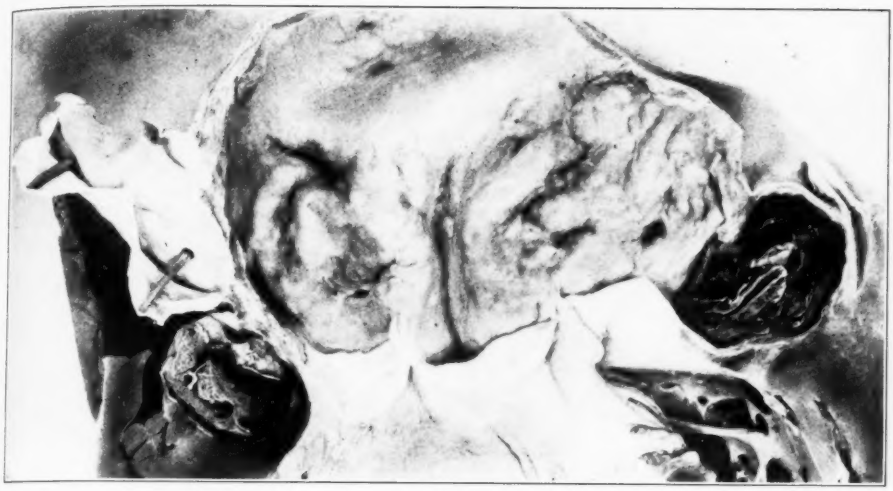
DESCRIPTION OF PLATES

PLATE 169

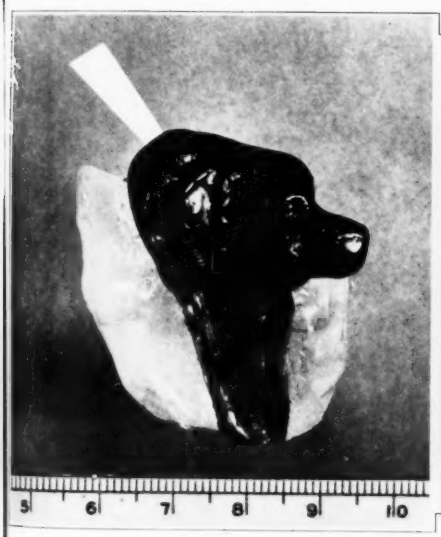
FIG. 1. Photograph of a portion of the left ventricle and aorta of Case 1. To the left are two right coronary ostia and a portion of the thrombosed Valsalva aneurysm. Above and to the extreme left are the right and left anterior pulmonic leaflets held out by props. To the right is the left coronary ostium and the Valsalva aneurysm showing its direct connection with the aorta and its roof formed by the beginning of the pulmonary artery. The groove to the right of the sac lies between the lateral wall of the pulmonary artery and the left anterior leaflet of the pulmonic valve.

FIG. 2A. Wax reconstruction of Valsalva (white) and left coronary (black) aneurysms viewed anteriorly. Natural size. A white marker has been placed in the ostium of the artery. To the right is the beginning of the circumflex branch. The flatly oval shape of the coronary aneurysm is well shown in this view.

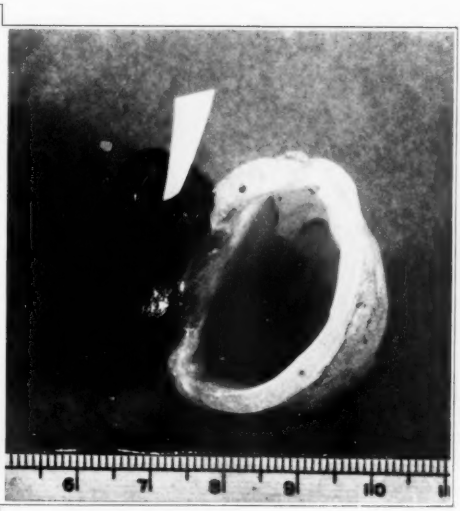
FIG. 2B. Wax model viewed from above showing the cavity of the Valsalva aneurysm. The white area toward the base of the coronary artery is a highlight.



1



2A



2B

PLATE 170

FIG. 3. Case 1. Drawing of the two aneurysms. Actual size. The distal face of each segment excepting Block 1 is depicted. Thus if H were superimposed upon G and so on, the aneurysms would appear as they were before sectioning.

FIG. 3A. Ostium of left coronary artery.
FIG. 3B. Block 5 mm. distal to ostium of left coronary artery.
FIG. 3C. " 11 mm. " " " " " " "
FIG. 3D. " 15 mm. " " " " " " "
FIG. 3E. " 20 mm. " " " " " " "
FIG. 3F. " 25 mm. " " " " " " "
FIG. 3G. " 30 mm. " " " " " " "
FIG. 3H. " 33 mm. " " " " " " "

a, ostium of left coronary artery; *b*, pulmonary artery; *c*, left atrium; *d*, mitral valve cusp; *e*, Valsalva aneurysm; *f*, left main coronary artery; *f'*, anterior interventricular branch of left coronary artery; *f''*, circumflex branch of left coronary artery; *g*, left ventricular myocardium; *h*, myocardial scars; *x x'*, and *x' y*, indicate boundaries of areas from which blocks for microscopic study were taken.

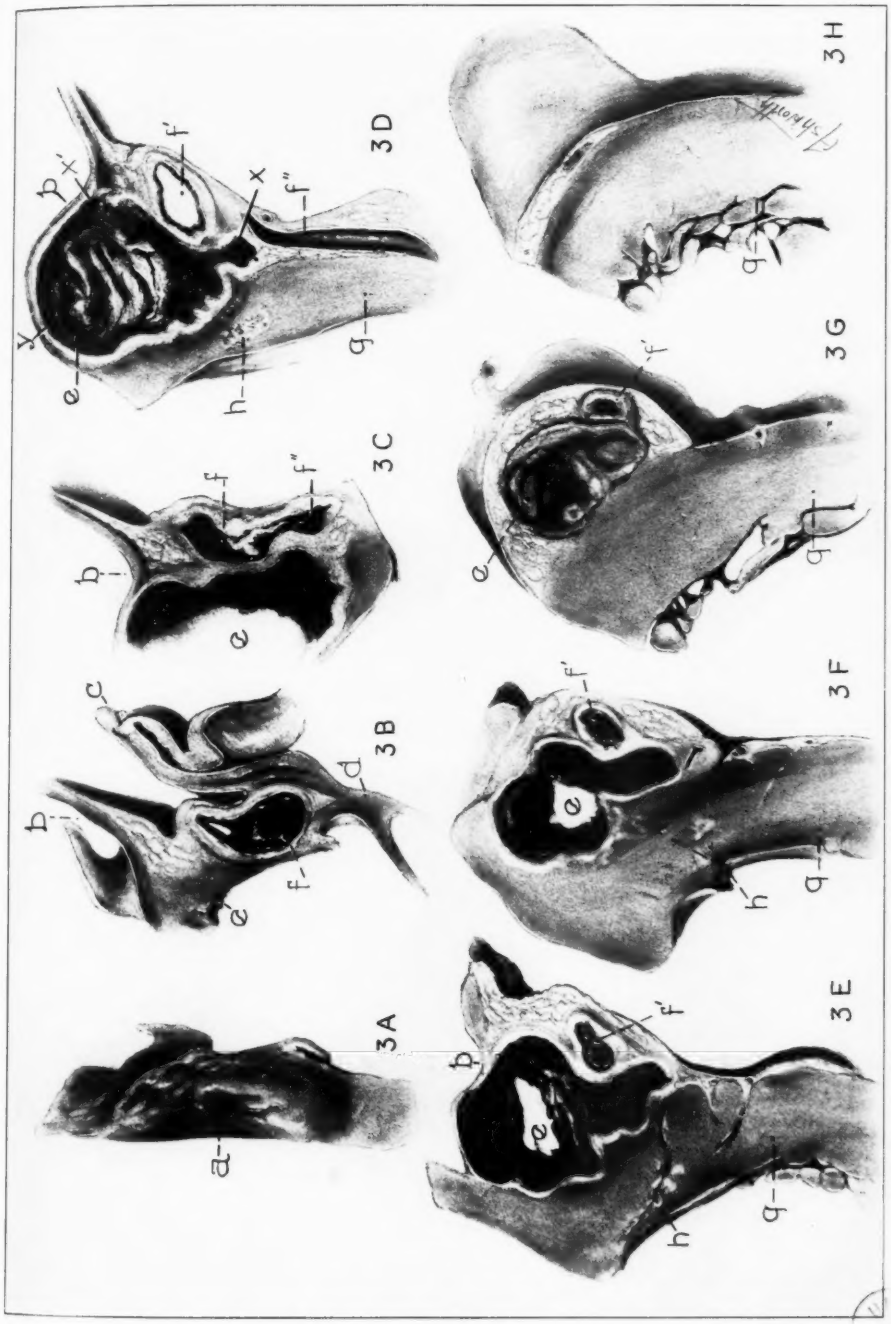
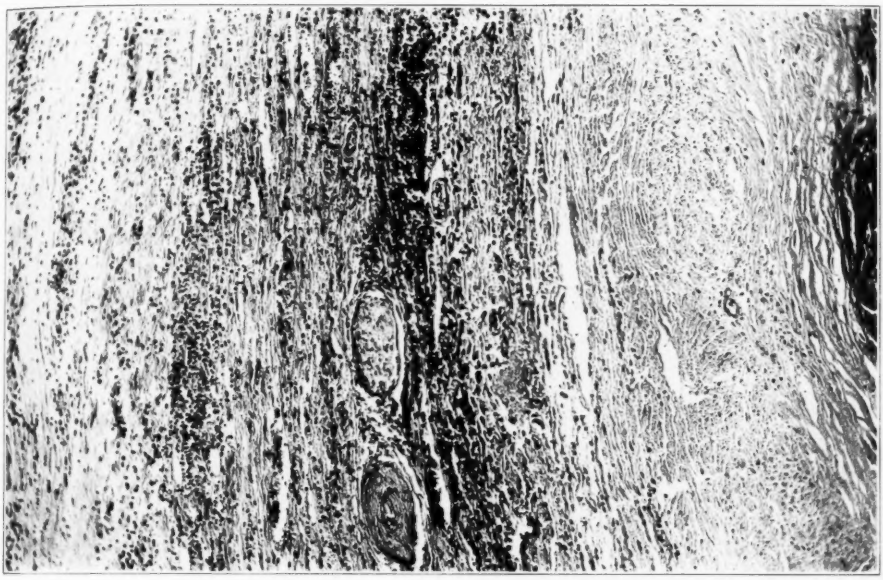

3A
3B
3C
3D
3E
3F
3G
3H

PLATE 171

FIG. 4. Case 1. Low power photomicrograph showing the intima, media and a small portion of the adventitia from the left coronary artery aneurysm near the point where it fuses with that of the sinus of Valsalva. Along the right margin is a small part of the occluding thrombus. The intima is thickened and contains a small capillary. Much of the media has been destroyed and is heavily infiltrated with lymphocytes and plasma cells. A nutrient vessel with marked narrowing of its lumen is shown and above it is a tangentially cut nerve. Note also the cellular infiltration and fibrosis of the adventitia at the extreme left.

FIG. 5. Case 2. View of the Valsalva aneurysm after opening the left ventricle and aorta. A part of the wall of the aneurysm has been cut away and turned to the right in order to display the bed of the sac in the interventricular septum. Near the left border of the aorta is the orifice of the left coronary artery. Note the thickening and distortion of the intima in the ascending aorta.



4

5

ADAMANTINOMA OF THE UPPER JAW *

REPORT OF A CASE

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Prior to the publication of Malassez' [1] work the grouping together of certain tumors of the jaws, as having a possible common origin in the gingivodental epithelium, may be said to date from the theory advanced by Verneuil (cited by Charvot [2] and Cumston [3]) that all growths of epithelial character in the jaws, especially multilocular cysts, adenomatous cysts and the periosteal cysts of Magitot,[4] arose in cell masses which he called "paradental débris." However, the fact that Malassez' theory was widely known in France before 1881 is evidenced by Cumston's reference to it. Certainly by that time Falkson,[5] in 1879, had advanced the theory of enamel organ origin and the hypotheses of Broca [6] and Magitot [4] had already been accepted by many authors. The first description of growths of this type is found in Scultet's Armamentarium Chirurgicum, 1655 (pages 222–228), and the term adamantinoma was first suggested by Goebal [7] in 1897, and reintroduced by Ferrero [8] in 1906, to denominate tumors composed of a fibrous stroma and epithelium arising from that of the gingivodental tract. In this way the term has been used for growths such as those reported by Jeannel,[9] Péan,[10] Brown[11] and others, where only epithelium of the malpighian type was present and no trace of true adamantine cells could be found. In many instances, however, its application was limited to epithelial growths where no adult dental tissue could be detected. Coryllos,[12] and many French authors, on the other hand, have used it to cover all epithelial growths originating in the gingivodental tract irrespective of their contents. It is doubtful whether there exists any basis in fact for separating "dental cysts," that is, cysts in association with an erupted tooth, and "dentigerous cysts" (in which a non-erupted tooth is included in a growth of this type) from the

* Received for publication June 21, 1934.

group of adamantinomas proper, the former as inflammatory proliferations of the epithelial débris of Malassez, and the latter as of follicular origin. Coryllos [13] reports the occurrence of a growth of typical differentiated adamantine cells in a patient who had suffered no dental trouble prior to the age of 35 years, at which time a carious molar was extracted, followed by the development of the tumor. Both the temporary and permanent dentitions had been normal and complete. Origin by proliferation of the paradental débris of the affected tooth appears most probable in this case. Heath [14] states that so-called dental cysts may develop into multilocular, or even solid adamantinomas, if not completely extirpated. In the Report on Odontomes [15] is found the statement that, in the case of all such cysts, to avoid recurrence the entire epithelial lining must be destroyed. Kegel,[16] and others, believe that in such instances of either a multilocular or solid adamantinoma it is the presence of the growing tumor of dental germ origin that is responsible for the dental crises, and consequently for the tooth extraction, and that it is not irritation of the paradental débris by inflammation and suppuration, and later damage to the débris during extraction, that is responsible for subsequent tumor development. They cite in support of this the fact that fluid frequently escapes at the time of extraction. The fifth case recorded by Charvot would appear to refute this theory, as the patient insisted that at the time of extraction of the offending tooth no fluid escaped, although 6 months later, when an obvious swelling had developed at that site, fluid containing cholesterin crystals was drained from what proved to be a cystic adamantinoma in the tooth socket.

Malassez and Coryllos affirm that an adamantine growth formed around an unerupted tooth the root of which is developed, originates not in the follicular epithelium but in the gubernacular portion of the paradental débris (probably the remains of the cord of the enamel organ), by an excessive proliferation and cystic degeneration, which normally would bring about the eruption of the tooth. In the case of the permanent teeth, with the exception of the first molar which erupts like the deciduous teeth, malformation of the *iter dentis* or bony canal by which the tooth erupts may be the irritating factor, as first suggested by Albarran [17] (Fig. 2). Certainly in the Report on Odontomes it is stated that in some cases of dentigerous cyst "absence of the corresponding tooth has been assumed without reason,"

and that where a perfect tooth was included its root was as a rule embedded in the cyst wall. Coryllos points out that were such tumors of follicular origin the tooth might be expected to lie entirely within the cyst cavity. He further affirms that in the only true dentigerous tumor of follicular origin the crown only of the included tooth is formed, the tumor originating in the epithelium of Von Brunn's sheath, the sole portion of the enamel organ remaining after development of the tooth crown. Cases such as those reported by Remy [18] and Duret,[19] where the included wisdom tooth was covered by Nasmyth's membrane, so that all epithelial elements of the dental follicle were intact, may be quoted in support of this theory. Nor are such growths always of predominantly cystic character.

The classification of Coryllos, including as adamantinomas all jaw growths of dental origin, with a persistent epithelial factor, irrespective of the contents, would appear to have the merit of simplicity and logic. The only point to which exception may be taken is the inclusion of growths of purely malpighian type of epithelium exhibiting epithelial pearls and areas of cornification. Both the inner and outer layers of the enamel organ are formed by basal cells (Fig. 1), and one may therefore assume that the basal cells of the gingivodental tract may undergo one of two forms of evolution — stratification, as in the formation of gingival mucosa, or cylindrization and enamel secretion. No evidence has yet been offered to show that cylindrical ameloblasts may be transformed into malpighian cells. If the term adamantinoma is to be limited to growths exhibiting epithelium in the line of development toward enamel formation, tumors of the purely malpighian type, arising as they must in certain sections of the paradental débris, may better be termed "paradental acanthomas."

Most writers agree with the statement of Bloodgood,[20] that conservative operations tend greatly to increase the rate of growth and enhance the penetrative capacity of these neoplasms in the recurrences (stated by Kegel [16] to follow in 76 per cent of cases, and reported by Simmons [21] as following in every one of 10 cases in which an incomplete operation was carried out). However, it is of interest to note that Hautant,[22] in reporting a case of adamantinoma of the upper jaw involving the middle meatus and the maxillary antrum, regrets that he did a maxillary resection, since he did not take into

consideration the relation of the growth to that of a nasal polyp which had been removed previously and reported as being of adamantine structure. Lemaitre, in the discussion which followed, agreed with this opinion that removal of the cyst and opening of the antrum would have sufficed. He states that as true adamantinomas are met with "in the lower jaw only," he regards the growth as a degeneration of a "paradental cyst."

Bloodgood further affirms that even after a period of 29 years, if not subjected to previous operative interference, these growths will yield to radical operation. In the upper jaw, however, the early involvement of important structures makes adequate early intervention essential. The case of Santy [23] is one of considerable interest in that a solid growth of the lower jaw recurred in cystic form 6 years after resection of the jaw. Even then, no adenopathy was present.

Solid growths may grow as fast or as slowly as cystic tumors. In the congenital cases of Coote [24] and Massin,[25] both children were seen at the age of 6 months, at which time one of the solid growths in the latter's case had grown to a diameter of 1 inch. The cysts in Coote's patient, which were accompanied by suppuration, were apparently of appreciable size. Gentsch [26] asserts that cystic degeneration, which he believes is due to defective nutrition, is induced by pressure upon the tumor and points out that in his own case the solid part of the growth was that over the face. He explains the apparently rapid increase in size of the cystic type as due to their relative bulkiness. With Siegmund,[27] Jäger,[28] Hammer,[29] Papayoannou [30] and Séneque,[31] Gentsch believes that the connective tissue also takes part in the cyst formation. In the author's case also this appears to be true.

According to Kronfeld [32] the presence of osteoclasts in the stroma indicates rapid resorption of bone, and so ready extension of the tumor. This is reported in the case of Chibret,[33] the epular case of Böhmig,[34] and that of Bozo and Lattes.[35] Of the 25 upper jaw cases reviewed by Gentsch 14 were described as cystic, 10 as solid, and 1 as questionably solid, showing that cystic growths are as common in the upper jaw as are solid tumors. Of a total of 110 cases of tumor of the upper jaw reviewed, the author found that 60 were stated to be cystic and 40 solid. Solid tumors of the lower jaw occur also, although less frequently, but many cystic cases, such as those described by Carter,[36] are reported as being partly solid. The

tumors reported by Nové-Josserand and Bérard,[37] St. Germain,[38] Derujinsky,[39] Nasse,[40] Chibret,[33] and Kruse [41] were all solid growths occurring in the lower jaw.

There is little doubt that solid growths are more likely to be treated by radical operation in the first instance, whereas cysts are often inadequately curetted, which probably explains Nové-Josserand's assertion that solid growths do not recur. There appears to be no constant relation between the consistence of these growths and their histology.

The histology of recurrences of these tumors also varies greatly. "Enamel" was present in a third recurrence of the polycystic type showing differentiated histology in D'Aunoy and Zoeller's [42] third case, while in the first case of Simmons, already quoted, the recurrence consisted entirely of undifferentiated cells. In the cases reported by Falkson,[5] Becker,[43] and Wright,[44] the recurrences showed differentiated histology. In the tumor of the upper jaw reported by Cordeiro and Cansancao,[45] after 15 years duration and frequent incisions the histology persisted in the differentiated form. There would appear to be evidence showing that it is possible for adamantinomas to develop malignant characteristics associated with three types of metaplastic change in the growths.

Stromal changes of a sarcomatous type have been reported by Eve,[46] in a tumor of the lower jaw in which enamel organ tissue and epithelium were present also. Fergusson's case, reported by Heath,[47] was that of a round cell sarcoma with metastases to the pelvis and biceps, after the cystic growth had lasted for 35 years. He described also [48] a case of adamantinoma showing this change. There were no enlarged glands.

The Report on Odontomes states that there is on record another case in a boy of 5 years, in which the dentition was complete. The growth contained the crown of a permanent third molar and the tissue resembled that of a sarcoma, and also "was not unlike dentin." All these growths occurred in the lower jaw. Mainguy's case [49] is probably also of this type, as he states that the growth seemed to him to arise in connection with the second left upper molar tooth, although the tissue was shown to be sarcomatous.

Epithelial changes apparently may be of two types, as might be expected from the nature and origin of these growths: (1) cylindromatous metaplasia, *i.e.* of the basal cell type alone, as reported

by Kaufmann,[50] Bozo and Lattes,[35] Bercher and Grandclaude,[51] Gernez and Surmont,[52] and others. In the case of Bercher and Grandclaude there was sudden increase in the rate of growth, with fracture of the bony wall associated with cylindromatous changes in the tumor. Raach,[53] in Case 3, describes a similar change, and Simmons[21] also, in a woman of 62 years. Séneque describes a tumor with a "cylindromatous stroma." (2) Squamous changes of a carcinomatous character occurring in growths of the epidermoid or acanthomatous type are reported by Suker [54] and Ewing.[55]

The sarcomatous changes in the stroma have been held responsible for the metastatic deposits reported by Eve,[46] Fergusson,[47] and Hutchinson,[56] while in Ewing's first case the metastases were definitely histologically related to the primary fibro-epithelial tumor in the jaw. In the second case, after the fourth recurrence, metastases to the lungs, neck and cervical lymph glands occurred. Simmons [21] also reports 2 cases in which metastasis occurred; in one the growth was of 14 years duration, and in the other 12 years. In Case 7 the metastatic deposit exhibited the same histology as the primary tumor, while in Case 1 it showed a more differentiated histology. Bernays [57] believes that constitutional weakness induces a malignant condition. In all these cases there was a long history with record of frequent operative interference.

The case of Vorzheimer and Perla,[58] in which there was removed postmortem from the right bronchus a mass consisting of tumor tissue composed of cylindrical cells, a few stellate cells and central masses showing a roughly formed epithelial pearl structure with numerous spindle cells enclosed in a fibrillar network, is of interest as the authors stress the fact that there was no evidence of a malignant condition in the tissue. The patient was a man of 38 years, in whom an upper jaw adamantinoma had been present for 21 years and who had undergone several operations, with radium treatment also. Following this a radical operation was performed, but the patient developed lung complications and died. The authors are inclined to the belief, based on the histology of the mass in the bronchus and upon the fact that no invasion of the bronchial walls had taken place, that the lung involvement was due to aspiration of tumor tissue into the bronchus and not to metastasis of the growth.

The following report of a case of adamantinoma of the upper jaw, occurring in an Indian patient, is presented in view of the

comparative rarity of the condition and also because of the peculiar points in the histology of the growth.

Report of Case *

A portion of a partly solid growth was received for pathological examination on April 5, 1933, with the following history:

"The patient, Mohd. Hafiz, is a Moslem youth of 18, admitted as suffering from a hard swelling of the left upper jaw, duration 1 year. The tumour is situated over the second molar tooth, appears to have been painless, and is not tender. The teeth are regular; the jaw appears to be expanded. The jaw was X-rayed and the film suggested the presence of cystic formation in the region of the tumour.

The growth at operation was found to be partly solid and partly cystic; it had invaded the antrum. The wisdom tooth which had erupted into the antrum was surrounded by solid tumour tissue. The tooth and tumour tissue were removed and the cavity curetted."

Unfortunately, it has not been possible to obtain the subsequent history of this patient.

Macroscopic Appearance: The tumor tissue was of firm consistence, reddish in color, and its cut surface exhibited the presence of minute cyst-like spaces, which varied in size from being barely visible, to 0.6 cm. in diameter. The tissue was encapsulated.

Microscopic Examination: The upper part of the growth is surrounded by fibrous tissue which forms a rough but distinct capsule. This consists of comparatively well formed connective tissue bundles somewhat irregularly arranged and exhibiting in places well marked myxomatous degeneration, resulting in the development of cystic spaces varying from 10μ to a size visible to the naked eye in diameter. Some are empty and others contain mucoid material. The nuclei in this region are scanty, well formed and stain homogeneously. The blood vessels are numerous and possess definite walls. More or less linearly disposed in the outer margin of this part of the growth are many fragments of osteoid tissue exhibiting well marked lacunae but no Haversian canals. The lower part of the fibrous capsule of the tumor is made up of connective tissue of a more homogeneous myxomatous character and of lighter texture, with fewer and less well formed vessels, which are mainly distributed along its outer margin. Here, too, an almost continuous boundary is formed by calcified material, similar to that mentioned above. In all instances there exists a narrow margin of firm connective tissue external to the

* This case is reported through the kind permission of Lt.-Col. Wilson, I. M. S., Civil Surgeon, Delhi.

calcified material. Figure 3 illustrates the general scheme of arrangement of the tumor.

The remainder of the growth consists of epithelium and cellular stroma present in varying proportions, and is irregularly lobulated (Fig. 4). In many places marked degeneration of the stroma has resulted in the formation of cystic spaces containing a delicate homogeneous acidophilic substance (A, Fig. 4). In other parts the spaces are empty, and elsewhere the stroma is of a highly cellular character, and here a degeneration of a different type appears to have taken place (B, Fig. 4). This material is granular, highly acidophilic, and in places where the stroma is in contact with the epithelium can be seen as irregular, rounded calcified masses composed of aggregations of similar granules. This substance differs from that shown in C, Figure 4, in that an eccentric prismatic structure is absent; it is of definitely granular character and exhibits greater affinity for the acid stain. It appears to be developed in and from the connective tissue only, but cannot definitely be interpreted as being poorly formed dentin. In one instance an entire cyst cavity was filled with this material, the granules varying in size but all more or less rounded in shape, while scattered along the margin of the cavity were larger masses of paler color, but of obviously the same origin. In places, it seemed that the material was being laid down on fine processes produced by change in the stroma.

The epithelial disposition is roughly lobular, the lobules varying in size and delimited by the highly cellular stroma. Many of the lobules are formed by masses of small epithelial cells of a roughly spherical shape, with scanty cytoplasm and large, somewhat pyknotic nuclei. No mitotic figures were seen, although in many instances the chromatin was diffuse. Alveolar distribution was also seen and the cells were of a low cylindrical type, regularly arranged on a basement membrane and flanked by one of two rows of flattened cells, thus reproducing the arrangement of cells of the inner and intermediate layers of the developing enamel organ. In several instances, without alteration in the peripheral row of cells, those of the central mass had begun to degenerate by vacuolization of the cytoplasm, so that the nuclei appeared to be connected by fine cytoplasmic processes only. In Figure 5 is seen a further development of this process. Here the marginal cells have assumed a roughly cylindrical form and are disposed at right angles to the basement membrane,

while the central mass of cells has undergone marked degeneration, resulting in the formation of stellate cells similar to those forming the enamel pulp in the 4 months fetus. It is noteworthy that these cells are in this case formed by degeneration of cells of the baso-cylindrical type and not of the malpighian type, as is seen in the developing enamel organ. Indeed, no malpighian cells were seen in any part of the tumor. In Figure 3 B an early stage of this degeneration is seen.

Further stages of evolution of the epithelial structure are seen in Figure 5 and at D in Figure 4. These consist in the development of regular alveolar spaces formed by a single or double row of high cylindrical cells with well staining homogeneous or slightly pyknotic nuclei disposed toward the cavity or lumen. At the outer margin can be detected a row of supporting flattened cells delimited by a well staining basement membrane. A distinct membrane lines the alveolus and can be seen clearly in Figure 4. This resembles the preformative membrane of the enamel organ, and is similar to that described by Siegmund [27] in a case of adamantinoma. Attached to the inner margin of this structure are fragments of a substance that appears to be produced by the epithelium and a mass of which lies free in the alveolar space (C, Fig. 4). From its structure it appears to be built up of successive layers of roughly hexagonal prisms which are highly irregular in size and distribution, having a vague resemblance to enamel.

At one point in the outer margin of the tumor an interesting development can be seen (Fig. 6). Here there is a narrow, finely moulded rim of material similar to that seen at C in Figure 4. Arranged over it is a well defined arch of a substance consisting of fine prisms closely arranged so as to give the appearance of canaliculi, with a central plaque of homogeneous calcified material. Covering this and extending outward into a somewhat uneven and indefinite margin is material of a similar character but of lighter texture. It is seen to be disposed over the substance shown at B in Figure 4. In no other part of the tumor are these substances seen in direct contact, and in all other instances the enamel-like material is contained within an alveolar space. In further support of the statement that the material shown at B in Figure 4 is formed solely in the connective tissue is the fact that in one place it definitely assumes the place of the connective tissue, which at that situation forms a

dense, membrane-like support to material seen at C in Figure 4, and which prior to assuming the calcified form is seen in bead-like formation arranged along the fibrous structure. Finally, a mass of material resembling in every detail that shown at B in Figure 4 is formed.

The structure of the growth leaves no doubt that this tumor is an adamantinoma containing calcified tissue of three types, whose characteristics are difficult to interpret absolutely from the structure exhibited. The disposition of the enclosed molar tooth suggests a gubernacular rather than a dental germ origin, as does also the irregular distribution of two of these calcified tissues (B and C, Fig. 4), while their presence in both true and reversed order (Figs. 4 and 6) is highly significant.

The only record which could be found of an adamantinoma in an Indian patient is that of Tirumurthi.[59] The tumor in this case, a female patient, was situated in the lower jaw and weighed 3 pounds at the time of operation.

In the Report on Odontomes the following figures are given:

Dental Cysts: Found equally in both sexes; a total of 18 records were found, with 50 per cent of these occurring in the upper jaw. In 5 of these upper jaw tumors the tooth involved is stated—incisor 1, molar 1, premolar 3.

Dentigerous Cysts: Eighty-four cases, of which in 76 the sex was stated; 39 occurred in women and 37 in men. Forty-one were upper jaw cases; 30 involved the canine teeth, 16 the incisor teeth, 13 the third molars, 12 the first or second molars and 11 the premolars.

Multilocular Cysts: They state that all of 39 cases were lower jaw tumors.

Gentsch collected 24 cases with tumors of the upper jaw from the literature and reported 1. Cordeiro and Cansancao collected 27 cases from the literature, and reported 1.

The following cases have been traced by the author:

Juxta- or Pararadicular Adamantinomas (Dental Cysts): Twenty-three cases, in 20 of which suppuration was a feature; in the remaining 3 the patient attributed the growth to trauma.

Simple Adamantinomas: Fifty cases.

Epular Adamantinomas: Weinlechner[60] 2, Böhmig[34] 1, Raach[53] Cases 2 and 3 (described as "mixed tumours of the palate"). Probably also the bilateral growths reported by Lantier[61] were of this

nature. Wohl [62] described an adamantinoma of the upper lip close to the junction with the gum. Moulonguet and de Lambert [63] offer evidence to show that congenital epuli are of the nature of adamantinomas.

Dentigerous Adamantinomas in which the Enclosed Tooth Was an Unerupted Tooth: In 12 cases a canine tooth alone; in 3 cases the wisdom tooth alone; in 3 cases an incisor tooth; in 3 cases the first or second molars; and in 3 the tooth is not named. The records of the cases reported by Wrede and by Ricke were not obtainable, but Gentsch refers to the former author as not stating whether the wisdom tooth had erupted or not, and in Wrede's case no report was made as to whether the tooth was an unerupted or supernumerary one.

The cases in which the canine tooth was enclosed are those of Cumston,[3] Heïdé,[64] Gurd,[65] Mayet,[66] Delie,[67] Broca,[6] Nélaton,[68] Bayer,[69] Gensoul,[70] Crocquefer,[71] 2, Chompret and Dechaume,[72] 2.

The wisdom tooth was enclosed in 2 cases of New,[73] that of Tellier,[74] and in the author's case.

The incisors were found enclosed in the cases of Syme,[75] Salter,[76] and Vitalis.[77]

The first or second molar was enclosed in the cases of Jourdain,[78] Ollier,[79] Lucas [80] and Jay.[81] D'Aunoy and Zoeller [42] do not report the tooth enclosed.

The following cases must be considered separately for various reasons:

Coleman [82] reports a case in which a growth appeared at the age of 12 years, 28 dental corpuscles were present. Nine were single, each with a formed conical crown, 6 had many points, and there was present also an irregular dental mass. In the right upper jaw the canine premolar and first molar tooth were missing.

Tellaider [83] reports a case in a woman of 27 years, in the right upper jaw of whom were missing the first molar, both bicuspids and the canine tooth. One of these teeth erupted a year after the operation. At the age of 12 enlargement of the jaw commenced, later followed by infection. In the tumor were found 9 single teeth and 6 tooth masses with a covering of enamel.

The first case of Chompret and Dechaume, already cited.

The case of Banns,[84] in which an unerupted canine was present in one antrum and a molar tooth in the other.

Case 1 of Bayer, in which the growth contained 2 teeth, 1 of which was in the antrum. Both were canine teeth, one a temporary and the other a permanent tooth.

Dupuytren and Bransby Cooper are cited by Heath as each reporting a case of adamantinoma associated with an inverted tooth, but the tooth is not named.

The case of Morault,[85] in which a growth in the region of the left lateral incisor contained a malformed canine tooth astride the unerupted incisor, a condition which suggests that malunion of the halves of the canine tooth germ may account for such cases.

Many rudimentary teeth were present in the cases of Hildebrand,[92] and of Gilmer [86]; the former was in a child of 8 years, and the latter of 6 months duration in a boy of 14. In the former instance some 200 teeth were removed and in the latter 78.

In the case of Rousseau-Decelle and Crocquefer [87] the teeth numbered 15 and each was possessed of a separate pulp cavity.

Miller's [88] case is also of interest. In a child of 1½ years a growth of 8 months duration showed the presence of widely scattered immature permanent teeth.

In the case of Crocquefer (Case 2) in addition to the missing permanent canine tooth there was found in the tumor 1 normal tooth and 12 tiny teeth, surrounded by a pericoronary sac.

In Crocquefer's first case, in a man of 60 years, the tumor extended from the absent canine to the maxillary tuberosity, involving the entire floor of the sinus. Two small granulomatous nodes on the floor of the sinus were shown to be adamantine in structure; they were separated from the cystic portion of the growth which was lined by epithelium.

In the case reported by Cordeiro and Cansancao [45] cement was found.

In the course of a discussion in connection with the report of cases by New,[73] Teter stated that he had seen several cases of multilocular cysts of the upper jaw, some containing enamel and cementum.

The case of Pedrescu-Rion,[89] in which enamel was present in a recurrence of an upper jaw growth, is of considerable interest, especially as the patient gave a 4 plus Wassermann reaction.

The case of Reverdin [90] and that of Jeannel,[9] already cited, are apparently of the nature of solid acanthomas.

It is possible that better drainage afforded in cases of upper jaw sepsis has some influence in reducing the number of adamantinomas met with in the upper jaw, as there is no doubt that dental cysts occur at least as frequently there as in the lower jaw, and according to Gentsch and others, cystic development in these growths is facilitated by defective nutrition and pressure, both of which factors are closely related to infection. The fact that these tumors appear to be associated with unerupted teeth, equally in either jaw, supports this hypothesis also. Developmental defects, as affording mechanical interference with eruption, may play an important rôle in the incidence of adamantinomas connected with unerupted canine and incisor teeth, as suggested by Chaminade [91] and Kegel.[16] It has been shown that entirely solid tumors are rare in either jaw, but that in the upper jaw, while probably a higher proportion of more nearly solid tumors is met with than in the lower jaw, predominantly cystic growths are at least as frequent as the more solid variety.

Conclusions

1. There appears to be insufficient evidence on which to separate "dental cysts" and "dentigerous cysts" from the group of adamantinomas proper.

2. As the basal cells of the gingival epithelium of the fetus appear to be capable of evolution in two directions, either toward the formation of typical malpighian and stratified epithelium or toward "cylindrization" and enamel secretion, the growths of purely malpighian cells had better be separated from the group of adamantinomas; the term suggested for them is "paradental acanthoma."

3. Gross structure and histology cannot be regarded as an index of rate of growth, possibility of recurrence, or malignant changes in the adamantinoma.

4. Malignant metamorphosis in these tumors may be of three kinds: (1) sarcomatous change in the stroma; (2) cylindromatous; or (3) squamous carcinoma changes in the epithelium. This tends to bear out the theory of two distinct non-interchangeable forms of evolution of the basal cells.

5. There is some evidence to show that better drainage of septic dental conditions may be responsible for the greater rarity of adamantinomas in the upper jaw.

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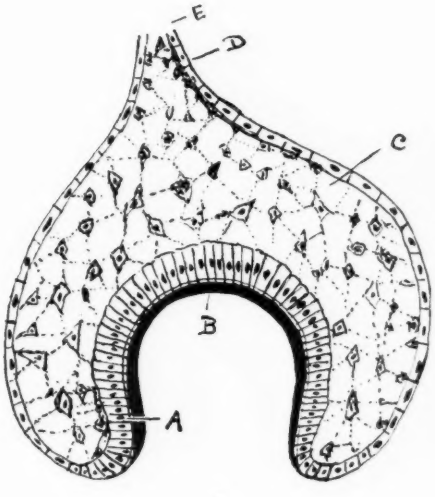
DESCRIPTION OF PLATES

PLATE 172

FIG. 1. Diagram of the enamel organ at the 5th month. A = enamel-forming cells; B = enamel; C = stellate cells of the enamel pulp; D = external epithelial layer; E = cord of the enamel organ. The preformative membrane lies between A and B.

FIG. 2. A transverse section through the lower jaw passing through the temporary and permanent molars in a child of 3 years (Malassez and Galippe [1]). A = iter dentis; B = gubernaculum dentis; C = temporary molar; D = permanent molar; E = gum margin.



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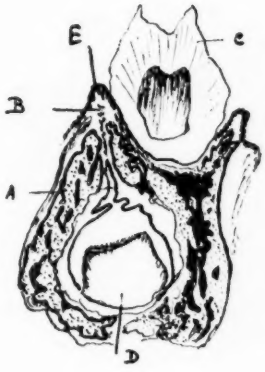

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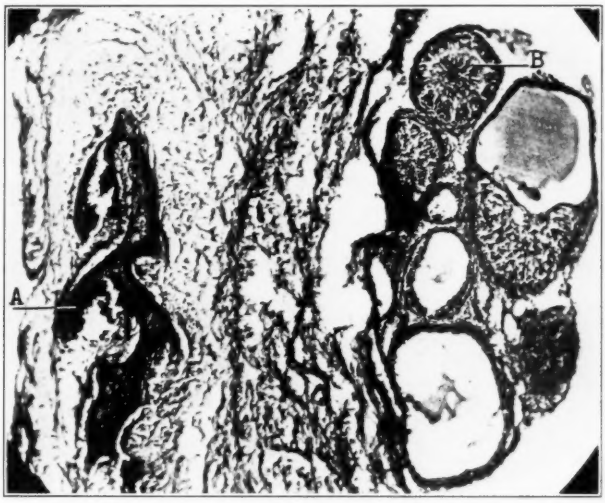
PLATE 173

FIG. 3. Illustrating the distribution of the stromal and epithelial elements of the tumor. A = osteoid tissue resembling cementum; B = formation of stellate cells.

FIG. 4. Showing the various epithelial forms present in the growth. A = stromal degeneration, acidophilic in reaction; B = the substance formed in relation to connective tissue only suggestive of poorly formed dentine; C = the substance apparently derived from the epithelium and of roughly prismatic structure; D = the high cylindrical epithelial cells.



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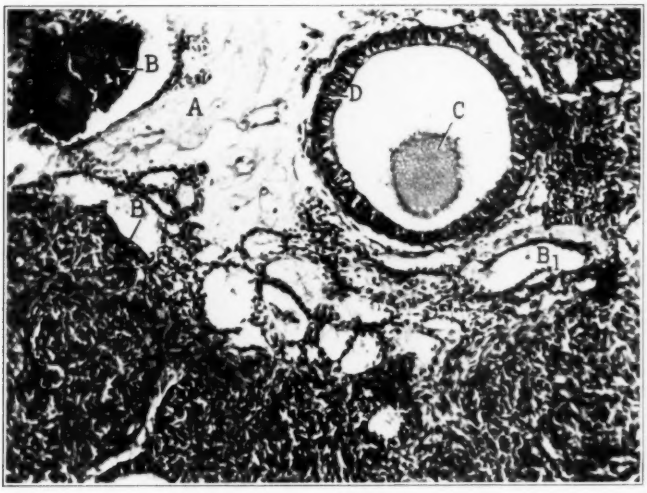

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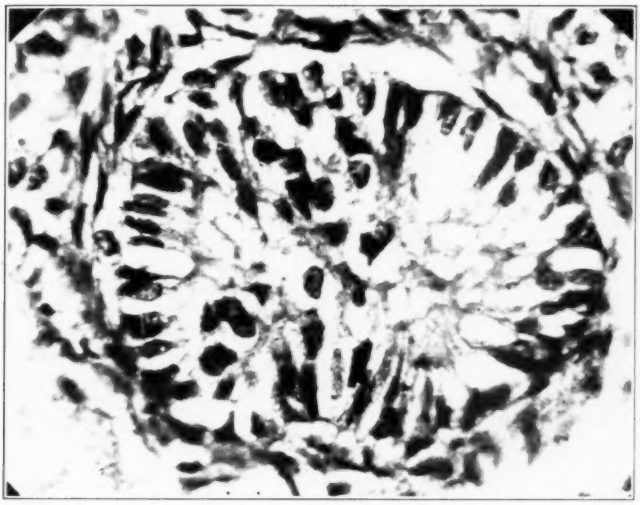
PLATE 174

FIG. 5. Showing the production of the substance shown at C in Fig. 4, by degeneration of the epithelium.

FIG. 6. The relation of the two types of calcified material at the margin of the growth, but within the capsule. A = the dentine-like substance; B = the finely prismatic structure; B_1 = a less well formed stage of the same substance.



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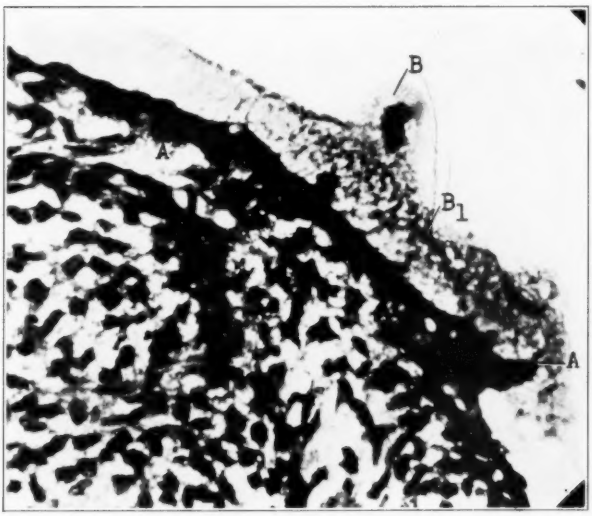

6

CONGENITAL ATRESIA OF THE TRICUSPID ORIFICE AND ANOMALOUS ORIGINS OF THE CORONARY ARTERIES FROM THE PULMONARY ARTERY *

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A case of atresia of the tricuspid orifice with associated interventricular septal defects and patent foramen ovale, and with anomalous origins of both coronary arteries from the pulmonary artery, has recently been observed in this laboratory. Primary atresia of the tricuspid orifice is an uncommon congenital cardiac malformation. Abbott [1] mentions only 9 cases of this condition in a review of 850 cardiac malformations. In a more recent survey Breslich [2] has recorded a total of 13. Two more cases have been reported [3, 4] since then which, together with the present one, brings the total to 16. The origin of one coronary artery from the pulmonary artery has been reported [5, 6, 7] by several observers. A survey of the literature, however, yielded no instance where both coronary arteries arose from the pulmonary artery. In view of the rarity of the condition the following case is reported.

REPORT OF CASE

Clinical History: The patient, a white female infant, 10 hours old, was born in the New Haven Hospital on Jan. 18, 1934. The mother, a 26 year old multipara, entered the hospital 1 month before the expected date of delivery because of vaginal bleeding. The condition was diagnosed as placenta marginalis and a Voorhees' bag was inserted to control the bleeding. The child was delivered spontaneously 3 hours later at 10.00 P.M. The child cried and breathed spontaneously and, except for moderate cyanosis and low temperature, appeared to be in good condition. It was seen on several occasions during the night and appeared to be doing well. When seen at 7.00 A.M. on the following day it was markedly cyanotic and respirations had ceased.

At autopsy the child was well nourished and well developed. It appeared to be full term and weighed 2725 gm. The mucous membranes and the skin of the head, face and neck were deep purple. The

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significant anatomical findings, in addition to the malformations in the heart, were bilateral congenital pulmonary atelectasis and hemorrhage into the tentorium cerebelli.

Description of Heart

The heart *in situ* was globular and did not appear enlarged; its transverse diameter was 5 cm. and that of the chest at the same level was 8.5 cm.

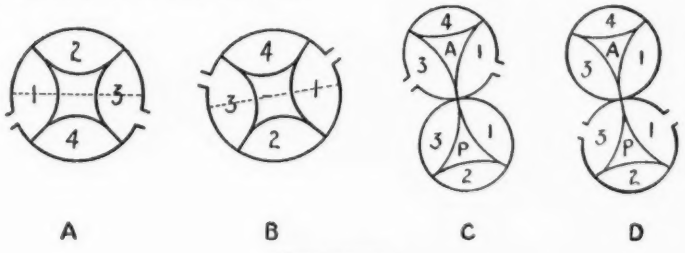
The pulmonary artery and aorta arose in the usual manner but showed a striking disproportion in their relative sizes. The former appeared to be about one-third the size of the aorta, which was of average caliber. One cm. from its origin the pulmonary artery divided into two vessels of equal caliber, each 1 mm. in diameter, one of which joined the aorta as the patent ductus arteriosus and the other continued as the left pulmonary artery. The aorta pursued its usual course but gave off from its ascending portion 1 cm. from the aortic ring a vessel 5 mm. in diameter which entered the hilum of the right lung. The vessels supplying the head and neck arose from the arch of the aorta in the usual manner. The distribution of the superior and inferior venae cavae appeared normal.

The heart weighed 19 gm. The apex was rounded and was composed entirely of the left ventricle. The right atrium was not dilated. The musculi pectinati were well rounded and the wall was not thickened. The foramen ovale, which measured 1 cm. in diameter, was covered by a membranous fold of endocardium, except for a slit-like opening 2 mm. wide along the anterior margin. The coronary sinus opened into the right atrium at the usual site through an orifice 4 mm. in diameter. There was complete absence of the atrioventricular orifice. A pit-like depression in the thick muscular septum between the right auricle and ventricle marked the site of the tricuspid valve. No vestiges of the valve cusps were present. The right ventricle was an aplastic structure whose wall measured 1 mm. in thickness. Two circular defects, each 2 mm. in diameter, were present in the interventricular septum. One was situated in the membranous portion, and the other about 8 mm. directly below it in the muscular portion. The pulmonary artery arose from the conus arteriosus as a thin-walled vessel whose circumference was 1 cm. The three semilunar cusps were thin and delicate. The coronary arteries arose from the sinuses of Valsalva behind the two posterior cusps, the right cor-

onary from the right posterior sinus, and the left coronary from the left posterior sinus. The subsequent course and distribution of each of these vessels was normal. The left atrium was not dilated and its wall was not thickened. The only unusual feature observed here was an anomalous communication with the coronary sinus through a circular orifice 4 mm. in diameter situated on the posterior wall 5 mm. to the left of the foramen ovale. The left atrioventricular orifice measured 3.8 cm. in circumference. The leaflets of the mitral valve were thin, delicate and well formed. The left ventricle was markedly dilated and hypertrophied; its wall measured 4 mm. in thickness. The aorta arose from the ventricle as a large, well formed vessel which measured 2.4 cm. in circumference. The aortic valve was composed of three thin, delicate, semilunar cusps. No orifices were present in the sinuses of Valsalva behind any of these cusps.

Discussion

The relation of congenital atresia of the tricuspid orifice to abnormalities in the embryological development of the septa of the heart has been adequately described by Breslich,[2] who also has reviewed the literature on this subject.



Text-figure 1

Diagram showing the position of the distal bulbar swellings and coronary orifices before rotation, after rotation, and after division to form the pulmonary artery and aorta in the normal heart (A, B, C). D shows the arrangement of the coronary orifices in the present case. Modified after Feller.

The mechanism of the normal development of the pulmonary artery, aorta and coronary arteries is well presented by Feller,[7] the chief steps of which are shown in the line drawings in Text-figure 1. The truncus arteriosus with the four distal bulbar swellings and with the origins of the coronary arteries from 1 and 3 are shown in A. The

positions of these bulbar swellings after rotation through approximately 180° has occurred are shown in B. The subsequent fusion and division of 1 and 3 to form the pulmonary artery and aorta are shown in C. Thus, the coronary arteries normally arise in the forward half of the bulbar pockets 1 and 3, close to 4, which portions subsequently form the anterior cusps of the aortic valve. Should the coronary artery orifices instead arise in the posterior halves of 1 and 3, close to 2, which subsequently form the posterior cusps of the pulmonary artery, they would have the positions indicated in D, which apparently occurred in the present case.

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DESCRIPTION OF PLATE

PLATE 175

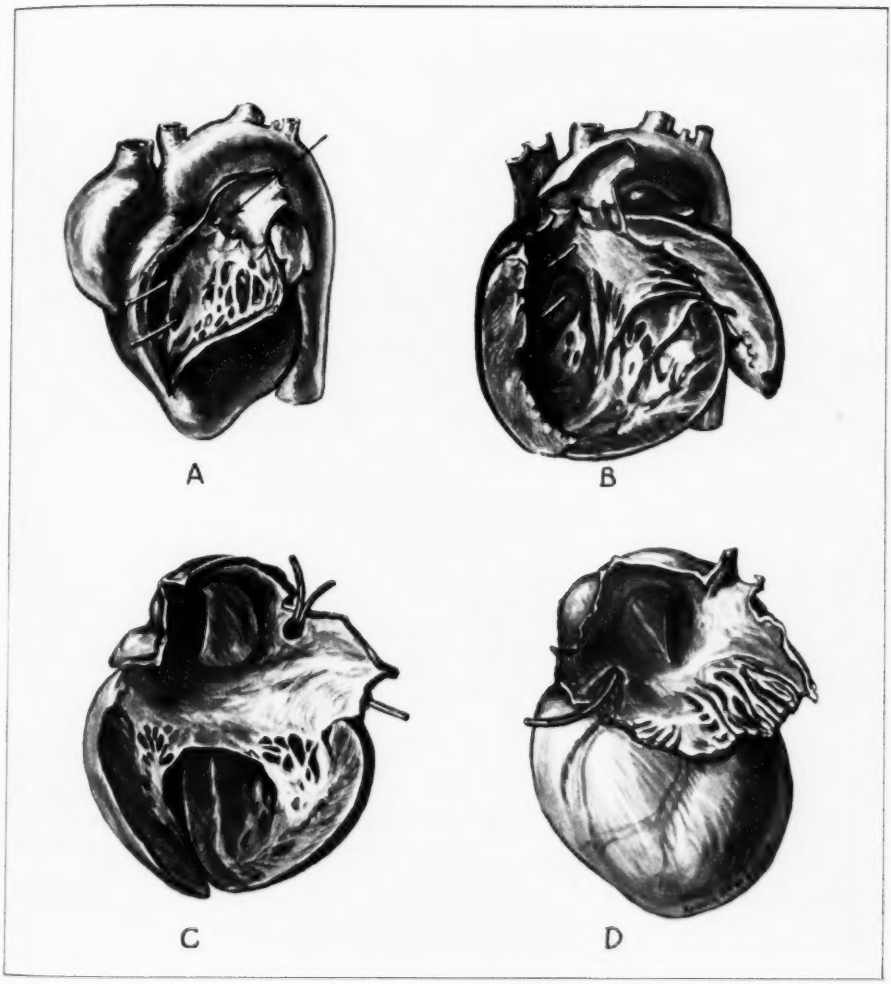
FIG. 1. (A 3038.) Atresia of tricuspid valve, with patent foramen ovale and interventricular septal defects; coronary arteries arising from pulmonary artery.

A = Right ventricle and pulmonary artery. Probes through septal defects. Orifices of coronary arteries in pulmonary artery. Aplastic right ventricle.

B = Left ventricle and aorta. Probes through septal defects. No coronary orifices in aorta.

C = Left auricle and left ventricle. Patent foramen ovale. Anomalous opening of coronary sinus.

D = Right auricle. Patent foramen ovale. Orifice of coronary sinus. Absence of tricuspid valve.


A
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CALCIFICATION IN THE BRAINS OF EQUIDAE AND OF BOVIDAE*

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In 1926[1] I estimated the frequency of so-called calcification in the vessels of the anterior half of the globus pallidus in man, and of "calcified" degeneration bodies there and in surrounding tissues. The condition had of course been described previously, but usually in connection with specific maladies, notably paralysis agitans and encephalitis lethargica; it now appeared that it must be considered as a phenomenon more or less normal in advancing years. Ostertag[2] has since recorded similar observations. In other territories of the central nervous system "calcification" occurs much less often, almost always only in connection with local pathological conditions; the most marked examples are perhaps to be found in some cases of porencephaly resultant from intra-uterine disease. Calcified "corpora amylacea" in the meninges are, however, of more or less normal occurrence.

While the mineral matter reacted strongly for iron, no definite evidence of the presence of calcium salts was forthcoming. Deposits of iron can give or obscure many of the color reactions commonly ascribed to calcium. Spatz[3] referred to the deposits as "Pseudokalk." Cameron[4] has studied very fully the staining properties of calcium salts and emphasizes the ease with which small amounts of iron are adsorbed from impure reagents, and so on; the globus pallidus is extremely rich in "free" iron (Spatz[3]) which might conceivably be concentrated in the affected vessels during life. At the time of my earlier publication I was not aware of the purpurin test for calcium, shortly afterwards used by Da Fano and Perdrau.[5]

This degeneration of the vessels of the globus pallidus is not confined to man. I have encountered somewhat similar appearances in even young monkeys (*Macacus rhesus*).[6] On the other hand, I have

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never done so in rabbits, guinea pigs, mice or rats. As the ensuing account will show, calcification in various forms occurs with surprising frequency in the equidae, and also in cattle.

Calcification in the Globus Pallidus

The brains of 16 horses, a Shetland pony and a mule were examined; none of the animals had a history of any illness other than the acute one to which it succumbed. Three under 7 years of age showed no calcification. Three (including the Shetland pony) aged 9, 9, and 10 years, respectively, exhibited calcified vessels in the globus pallidus, while one aged 11 years did not. The remaining 11 (including the mule) of ages between 13 and 25 years were all affected.

The histological appearances were in every way comparable with those in man. In different cases the deposits lay in the media of the vessels, in the adventitia, or in both. In some instances, relatively more frequent than in man, the affected vessels were almost obliterated by intimal thickening (Fig. 1), and the newly formed tissue also was on occasion lightly impregnated; these horses did not suffer from generalized vascular disease. A variable number of impregnated degeneration bodies were distributed similar to those in human cases.

In the brains of 2 of 7 cows (ages unknown) similar lesions were seen in the globus pallidus.

Calcification in Other Parts of the Brain

Two horses, aged 9 and 16 years respectively, exhibited finely granular deposits in the nervous tissue immediately adjacent to the adventitia of a few of the cerebral arteries. In one animal a half-dozen or so of the vessels in one part of the centrum semi-ovale were so affected; in the other two of the pontine vessels suffered. The mineral salts surrounded only a small part of the circumference of the vessels.

One horse, aged more than 20 years, showed most extensive lesions in the dentate nuclei of the cerebellum. Every vessel was heavily calcified and the tissues were strewn with calcified globules of all sizes (Fig. 2). Some of the capillaries were completely encased in sheaths of similar nature; considerable new formation of small vessels was apparent, and a degeneration of the nervous tissues with great reduction in the number of nerve cells. One large vessel had

apparently suffered thrombosis, with later recanalization; part of the artery wall and the remains of the clot were heavily impregnated with mineral salts (Fig. 3). These lesions possibly represented the aftermath of an acute process of unknown date and origin; the history of the horse prior to its fatal acute illness was not available.

The meninges and choroid plexuses of old horses frequently contain "corpora amylacea" which may or may not be calcified. In both young and older animals, in the former in the absence of changes in the globus pallidus, the larger meningeal arteries frequently show small calcified bodies lying usually immediately beneath the endothelium (Fig. 4). These corpuscles commonly consist of a larger laminated central body, roughly spherical or ovoid in shape, to which are attached several small outgrowths; their appearance may aptly be compared with that of a tortoise.

Staining Reactions of the Deposits

The deposits are blackened by the von Kossa method for detecting phosphates. They stain deeply with hematoxylin, purple or black according to the particular method employed. Tests for iron (Prussian blue reaction, Macallum's hematoxylin) are positive. Purpurin colors the degenerated areas purple, the color imparted to the dye when precipitated in the presence of iron salts. One hours immersion in 10 per cent oxalic acid prevents staining by the von Kossa or Prussian blue techniques; the areas now color red with purpurin, a reaction abolished by immersion in 5 per cent hydrochloric acid.

Thus, evidence of the existence of both calcium and iron salts in these deposits is forthcoming.

Summary and Conclusions

Calcification of the vessels of the globus pallidus is at least as frequent in middle-aged and old horses as in man at a corresponding period of life; it also occurs in cattle. In neither species can it be correlated with the pathological condition responsible for death. Since similar appearances are met with in monkeys, it seems probable that it may represent a biological phenomenon of some constancy in advancing life in the higher mammals. Unlike man, many horses, both young and old, show small calcified bodies in the intima of the larger

meningeal arteries. Calcification may sometimes be present in other parts of the central nervous system. The use of the purpurin test following treatment of sections with oxalic acid permits recognition of calcium salts in the presence of iron compounds; in the horse both are represented in the degenerated vessels.

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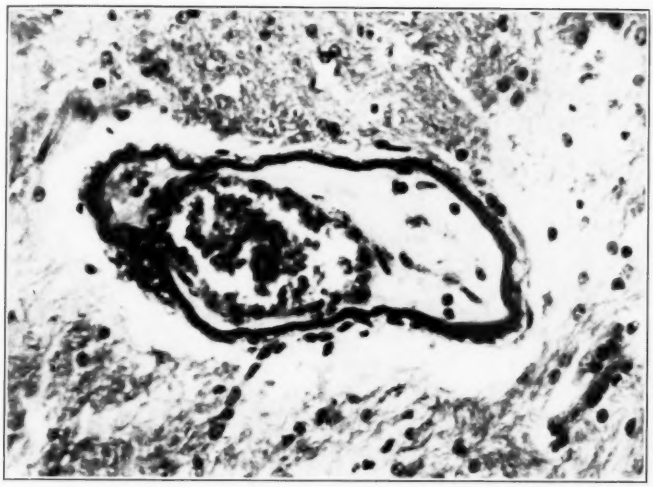
DESCRIPTION OF PLATES

PLATE 176

FIG. 1. Calcified vessel of the globus pallidus of a horse dying of equine encephalomyelitis, showing marked thickening of the intima. Iron alum hematoxylin and Van Gieson's stain. × 340.

FIG. 2. Vascular calcification and calcified globules in the dentate nucleus of the cerebellum in a horse dying of acute epizoötic leucoencephalitis (MacCallum and Buckley). Iron alum hematoxylin and Van Gieson's stain. × 215.



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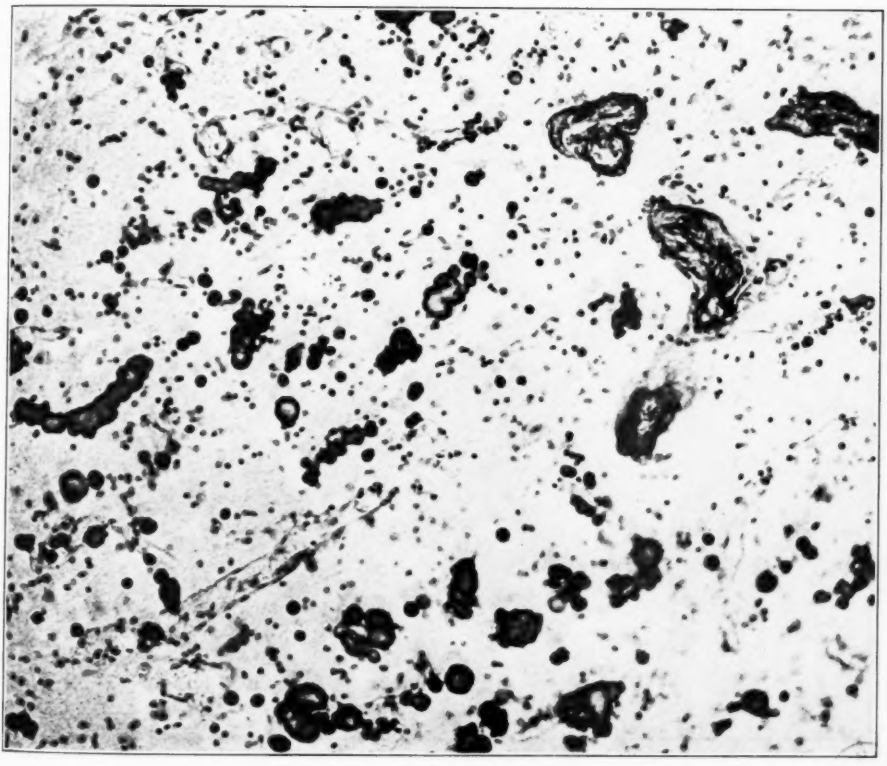

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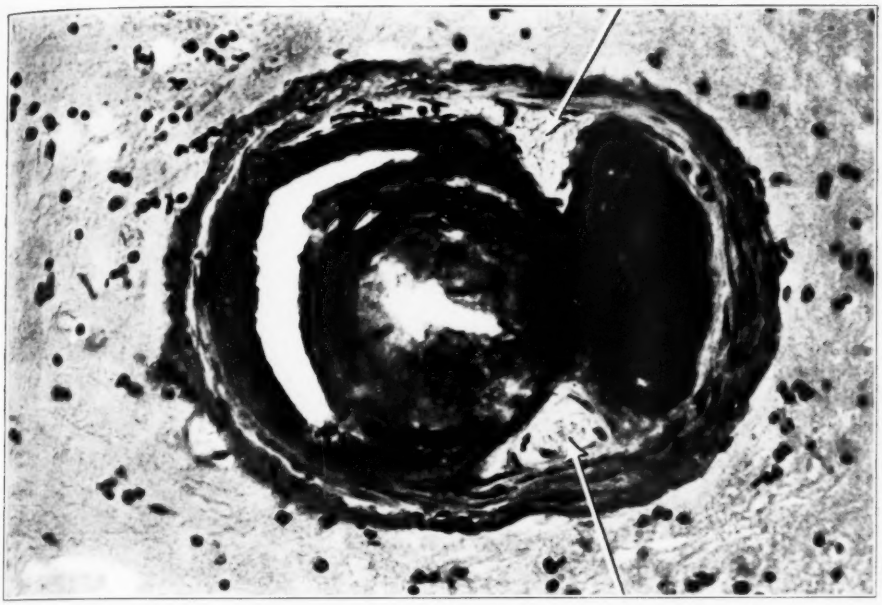
PLATE 177

FIG. 3. Calcified thrombus in a vessel of the dentate nucleus of the cerebellum; recanalization of the clot indicated by arrows. Same case as Fig. 2. Iron alum hematoxylin and Van Gieson's stain. × 365.

FIG. 4. Calcified bodies in the intima of a meningeal artery from a horse dying of forage poisoning. Iron alum hematoxylin and Van Gieson's stain. × 260.



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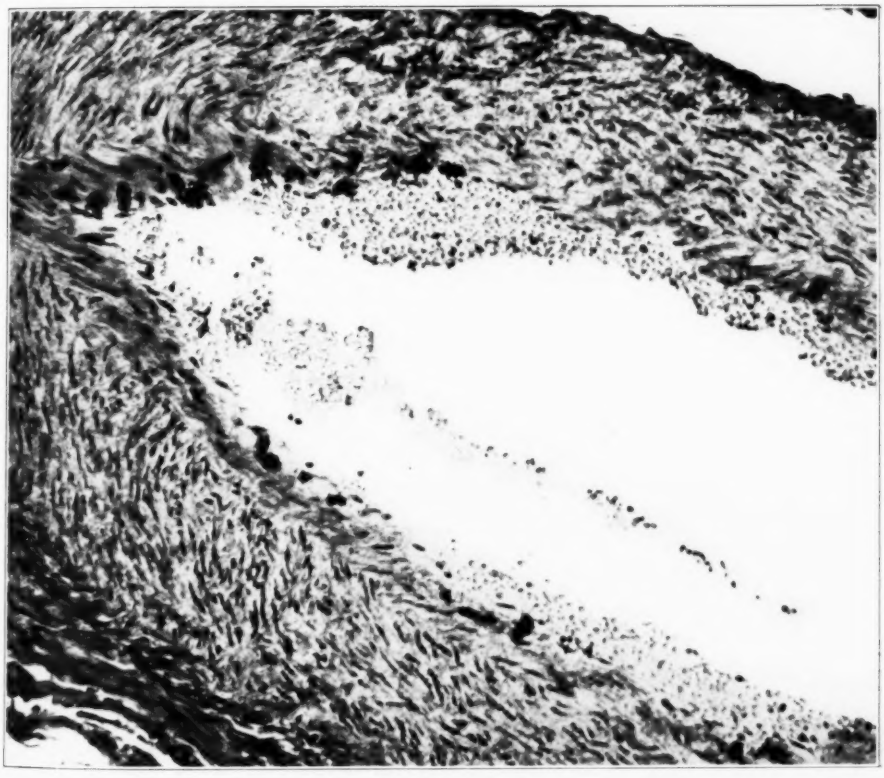

4

FOCAL FAT INFILTRATION IN THE LIVER *

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The statement that lipoma occurs in the liver is made in such textbooks as those of Borst,[1] MacFarland,[2] Adami and Nicholls,[3] Ribbert,[4] Stengel and Fox,[5] Delafield and Prudden [6] and Karsner,[7] but no mention is made by Schridde,[8] Ewing [9] and others. A critical search of the literature, however, fails to reveal any case or report that can be indubitably accepted as a true case of lipoma of the liver. Rolleston [10] stated that genuine fatty tumors do not occur in the liver and that what have been mistaken for these are small appendices epiploicae which have become detached from the colon and have come to rest between the diaphragm and the convexity of the liver. These are flush with the surface of the organ, but upon careful examination are found to lie outside the capsule of the liver. Turnbull and Worthington [11] described areas of atypical liver tissue under the capsule which are prone to fatty change, probably congenital anomalies. These changes, however, do not represent the size or appearance of the lesion to be described.

Because of the fact that there appears to be some doubt about the actual existence of lipoma of the liver and because the lesion about to be reported was first thought to be a lipoma, the following case is reported.

Report of Case

The patient, a 70 year old white female, died after surgical drainage of the gall-bladder. Autopsy revealed a scirrhous adenocarcinoma of the gall-bladder with local metastases to the liver, head of the pancreas and regional lymph nodes.

The liver weighed 1300 gm. The left lobe was relatively smaller than normal. The capsule was smooth and transparent and the edges were rounded. The color was normal. On the superior surface of the left lobe of the liver and directly under the capsule was a spherical nodule measuring 2 cm. in diameter. This was yellowish

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white in color, slightly raised above the surface, firm in consistence and not attached to the diaphragm. On section it greased the knife and on the cut surface the usual liver architecture was absent. The lesion was sharply demarcated from the surrounding normal liver as though it were enclosed within a capsule.

Microscopic Examination

A low power photomicrograph of the lesion (Fig. 1) stained with Mallory's aniline blue shows the outline to be somewhat irregular. There is no capsule. The only places where connective tissue can be recognized along the periphery are where the fat-like tissue abuts upon a portal space. Sections stained with sudan III reveal the fat to be of the neutral type.

Figure 2 shows the edge of the lesion in relation to the normal liver tissue. Here the infiltrating fat-like tissue can be seen in various portions of a lobule and again the absence of a capsule is apparent. The fat-like cells are of the adult "signet-ring" type and there is a suggestion of compression of adjacent liver tissue. The liver cells themselves show granularity of the cytoplasm but the nuclei are normal. There is a zone between normal and signet-ring cells in which liver cells contain small fat droplets. Along the periphery of the lesion and most particularly in abutting portal spaces there are occasional monocytes and lymphocytes. These cells are not present within the central portions of the lesion.

Within the center of the fatty lesion, but more clearly and with greater frequency along the periphery, small, intact bile ducts are present surrounded by a small amount of fibrous connective tissue and the cells containing fat.

Within the fatty area a moderate number of fairly large round cells with foamy cytoplasm and ovoid, deeply chromatic nuclei is found in the interstices between the liver cells infiltrated with fat. It is not known whether these cells represent young liver cells infiltrated with fat, or fixed or wandering histiocytes. The lesion is fairly well vascularized by large arteries and thin-walled veins and capillaries that appear to spring from the periphery. The arteries along the periphery, which seem to be derived from the portal spaces, show slight thickening of their walls and the veins for the most part are wide, thin-walled and gaping, although an occasional vein has a thickened wall.

Discussion

In 1925 Huguenin,[12] as quoted by Hanser,[13] described two types of focal fatty metamorphosis in the liver which had not previously been found in the literature.

Huguenin described one type of focal fatty metamorphosis occurring in the liver of healthy men and dogs and another type in men and animals dead of acute infectious disease. This latter type is found in the liver that is the seat of cloudy swelling.

The first type is found on the surface, is sharply delimited, although irregular in contour, and the diameter on the surface is at most 3 cm. and on the cut surface, at most, 1.5 cm. in diameter. Microscopic examination of the peripheral zones proves absolutely that the fatty metamorphosis is delimited by the borders of a lobule and that an individual lobule either is completely changed or not at all. The cells contain neutral fat. Further, there are changes in the vessels either within or without the focus and there is no evidence of inflammation, pigmentation or nuclear changes.

The second type is seen at autopsy in men and animals dead of infectious disease. Grossly these lesions appear exactly like the former type but microscopically show that the fatty metamorphosis is not contained within the borders of a lobule. Changes in the nuclei may be found and frequently lymphocytes are found between fat infiltrated cells. Huguenin believed that this latter type does not go on to complete recovery because of the nuclear changes and infiltration of lymphocytes, and that this type may end in circumscribed areas of cirrhosis.

Of the two groups, the lesion here reported should be placed in the second, although there are no significant nuclear changes and no infiltrations of lymphocytes within the fatty mass.

The lesion does not correspond to the usual picture of lipoma in that there is no trace of capsule and the supporting tissue is made up in considerable part of remnants of the capsule of Glisson. There are no features indicative of malignant neoplasm.

That the condition is a localized fat infiltration is supported by the fact that perilobular structures including bile ducts are found within the mass. There is no positive indication of marginal compression and between the normal liver cells and the fatty mass there is a zone, interpreted as transitional, in which many liver cells show

small fat droplets. The process differs from ordinary fat infiltration of the liver in that all the cells within the mass show almost complete distention of cytoplasm by a single, large fat globule.

Summary and Conclusions

A case of localized fat infiltration of the liver resembling lipoma in the gross and not identical with any similar lesion reported in the literature is reported. It is possible that other cases thought to be lipoma of the liver are of the same nature.

Note: The author wishes to express his thanks to Professor H. T. Karsner for the photographs.

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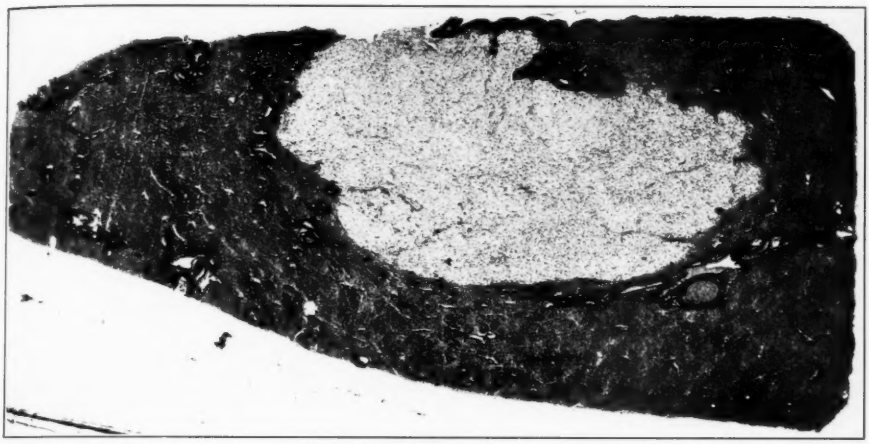
DESCRIPTION OF PLATE

PLATE 178

FIG. 1. Section showing irregular contour of the lesion. Mallory's aniline blue stain. × 3.

FIG. 2. Section showing lack of sharp definition of the lesion and lack of limitation to a single lobule. Hematoxylin and eosin stain. × 150.

FIG. 3. Small bile duct near center of main mass. Hematoxylin and eosin stain. × 820.



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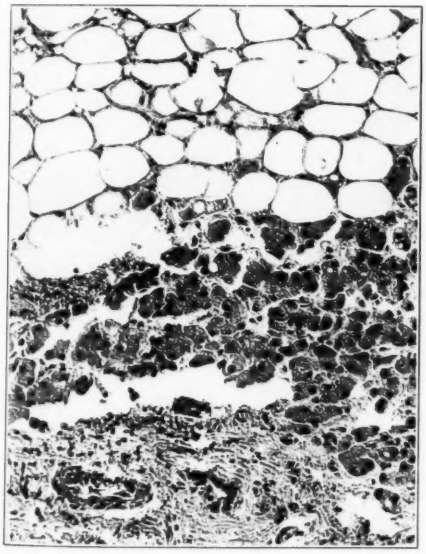

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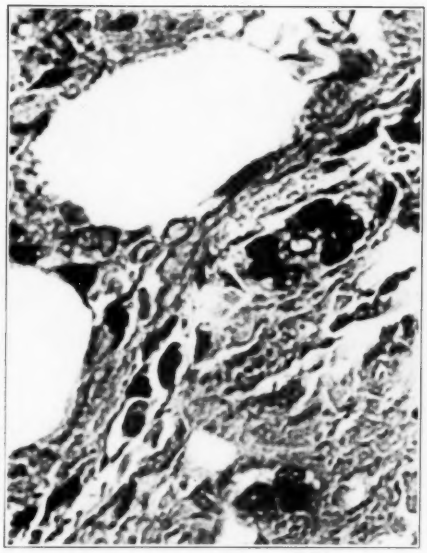

3

MENINGIOMA OF THE TUBERCULUM SELLAE WITH HYPEROSTOSIS *

REPORT OF A CASE WITH AUTOPSY FINDINGS

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The clinical syndrome produced by meningioma of the tuberculum sellae has been well formulated in the contributions of Holmes and Sargent,[1] who reported 10 cases, and of Cushing and Eisenhardt [2] with 15 cases. The typical course is described by Cushing and Eisenhardt as gradual impairment of vision in a person of middle age with bitemporal constriction of the visual fields, usually not equal in the two eyes, and primary optic atrophy. In the early stages these tumors do not deform the sella or cause secondary symptoms of hypopituitarism.

While about one-fourth of meningiomas situated under the vault of the skull produce thickening of the adjacent bone, in none of the cases of suprasellar meningiomas described by the above authors was hyperostosis detected. In one case Cushing and Eisenhardt [2] found a slight invasion of the tuberculum sellae by tumor cells. We present the following case of meningioma arising over the tuberculum sellae in which a marked involvement of the underlying bone occurred.

REPORT OF CASE

Clinical History: T. B., No. 64972, male, a 42 year old painter of Denver, Colorado, was admitted to the University of Chicago Clinics on July 31, 1932, complaining of loss of vision of 15 years duration. His past and family histories, so far as could be determined, were irrelevant to the present illness.

The patient first noted impairment of vision of the left eye in 1917, which grew progressively worse until it was reduced to light perception in 1920. Lead poisoning was suspected in 1922. In 1928 the vision of the right eye began to fail but the patient was able to do his work until 1930, at which time the left eye became totally blind. By 1932 vision in the right eye was reduced to the perception of bright light. A history of temporal constriction of the visual fields could not be elicited. There had been no headache or alterations in weight or strength.

* Received for publication June 11, 1934.

Physical Examination: The patient was a well developed, obese male, weighing 81.7 kg. An abundance of hair was present over the chest, axillae and pubis. Sense of smell was intact. The left eye was totally blind, while light perception was present in the inferior nasal quadrant of the right visual field. Both optic discs were intensely white with clear-cut outlines. A large area of lamina cribrosa was visible in each. The pupils were about 6 mm. in diameter with reaction to light only directly in the right eye and only consensually on the left. Ocular movements were full with a coarse lateral nystagmus on looking to either left or right. The tongue protruded slightly to the left. Sensation and strength were everywhere intact. Except for a moderate exaggeration of the left knee jerk and ankle jerk all reflexes were normal. The basal metabolic rate was minus 16 per cent.

Laboratory Data: The blood and urine showed nothing abnormal. X-ray of the skull revealed enlargement of the sella turcica with partial decalcification of the posterior clinoids. In oblique views taken for the optic foramina the tuberculum sellae could be seen to be greatly thickened.

A diagnosis of meningioma of the tuberculum sellae was made and a right frontal osteoplastic flap was reflected. During the course of extirpation of the tumor with the Bovie knife a large artery was torn and the patient died from hemorrhage.

Autopsy Report

Postmortem examination revealed an intradural hemorrhage, slight generalized arteriosclerosis, early nodular hyperplasia of the prostate, fatty infiltration of the liver, pancreatic rests in the duodenal mucosa, submucous urachus rests in the urinary bladder and a bile duct adenoma in the liver, in addition to the intracranial tumor.

A large tumor mass 4.5 by 4.5 by 2.5 cm. lay on the undersurface of the brain in the midline. It extended from within 5 cm. of the frontal pole posteriorly to the tuber cinereum and markedly compressed the inferior surface of the brain and the medial surfaces of the temporal lobes (Fig. 1). The tumor was very hard, grayish in color, and showed calcium deposits on gross section. The optic chiasm was greatly flattened over the superior surface of the tumor. The left optic nerve was completely obscured and the right markedly compressed by extension of the tumor into the optic canals. The hypophysis was flattened and compressed into the posterior part of the sella turcica.

A roughened bony eminence 2.5 cm. long, 2.5 cm. wide and 5 to 7 mm. deep occupied the site of the tuberculum sellae and posterior portion of the cribriform plates. The entire sphenoid was removed as a specimen. Section of this specimen in the midline showed the hyperostosis to be composed of dense spongy bone (Fig. 2).

Microscopic Examination

The posterior lobe of the hypophysis appears normal. In the center of the flattened anterior lobe the epithelial cells have disappeared and have been replaced by dense connective tissue surrounding an irregular central cavity.

The tumor itself is composed of elongated spindle-shaped cells with oval vesicular nuclei. The cells are arranged in fascicles and whorls, some of which are calcified, and which are separated by a rather extensive connective tissue stroma.

A decalcified section along the midline of the entire sphenoid reveals the hyperostosis of the tuberculum sellae to be composed of moderately thick trabeculae of bone with masses of tumor cells occupying the large cancellous spaces (Fig. 3). The architecture of the tumor masses is similar to that seen in the primary tumor, although none of the cellular whorls is calcified. A layer of loose fibrous tissue separates the masses of tumor cells from the bony trabeculae in most of the spaces. In some areas definite rows of osteoblasts are present, an indication that the new bone has been laid down by endosteal stimulation and not by the tumor cells directly. The tumor invasion extends inferiorly to the epithelium of the underlying sphenoidal sinus. The remainder of the sphenoid is essentially normal. Sections of the optic nerves show that the nerve fibers have been compressed and destroyed, not by the hyperostosis but by direct extension of the primary tumor down the optic canals.

Discussion

The idea, formerly held by some, that trauma plays a rôle in the production of hyperostoses (Spiller [3] and Penfield [4]) associated with meningiomas seems disproved by recent reports of such involvement of structures at the base of the skull, presumably removed from the effects of any but the most severe blows to the head. Winkelman in 1930 [5] and Stender [6] from this clinic have described cases of hyperostosis from meningiomas of the sphenoidal ridge. The present case demonstrates that another group of meningiomas of the base of the skull, the suprasellar meningiomas, may produce the same involvement.

The reasons are not clear for the apparent rarity of osseous involvement from meningeal tumors over the tuberculum. It is not unlikely, however, that tumors in this location, giving rise as they do

to serious loss of vision early in their course, cause the patient to seek surgical treatment earlier than do patients with meningeal tumors elsewhere. In the present instance the patient ignored the loss of vision which occurred for at least 15 years, during which time the tumor grew to tremendous size and had ample opportunity to invade the skull. It is also not impossible that small thickenings of the tuberculum may easily be overlooked, both in the ordinary X-ray views of the skull and at operation. In our case the only clear-cut clinical evidence for hyperostosis was in the oblique roentgenograms taken for visualization of the optic foramina. In the full lateral view the thickening of the tuberculum was not very evident.

The histological picture of the involved bone is similar to that of other meningiomatous hyperostoses. That the bony enlargement is a result of invasion of the cancellous spaces by tumor cells which stimulate osteoblasts to new bone formation has been demonstrated by Cushing [7] and by Phemister.[8] The latter has demonstrated further that a dense spongy arrangement of newly formed bone is characteristic of a slow rate of growth or a marked tendency to ossification. In the present case the trabeculae of bone were not as dense as in Phemister's cases, where the vault of the skull was the site of involvement. In connection with Phemister's statement that calcification in psammomas is in no way related to the bony thickening of the skull, it is interesting to note in our case that, while the primary tumor contained numerous calcified psammoma bodies, none was seen in the tumor masses in the hyperostosis of the tuberculum sellae.

Summary

A case of meningioma producing a hyperostosis of the tuberculum sellae is described. The history consisted of gradual loss of vision of the left eye for 15 years and of the right for 4. Examination revealed complete blindness of the left eye and only light perception in the inferior nasal quadrant of the right visual field. Bilateral optic atrophy was present. Death from hemorrhage occurred during operation. Autopsy revealed a large, globular suprasellar meningioma which invaded the optic canals and produced a marked hyperostosis of the tuberculum sellae. The primary tumor contained numerous calcified psammoma bodies, while the tumor masses invading the bone and stimulating hyperostosis showed no calcification.

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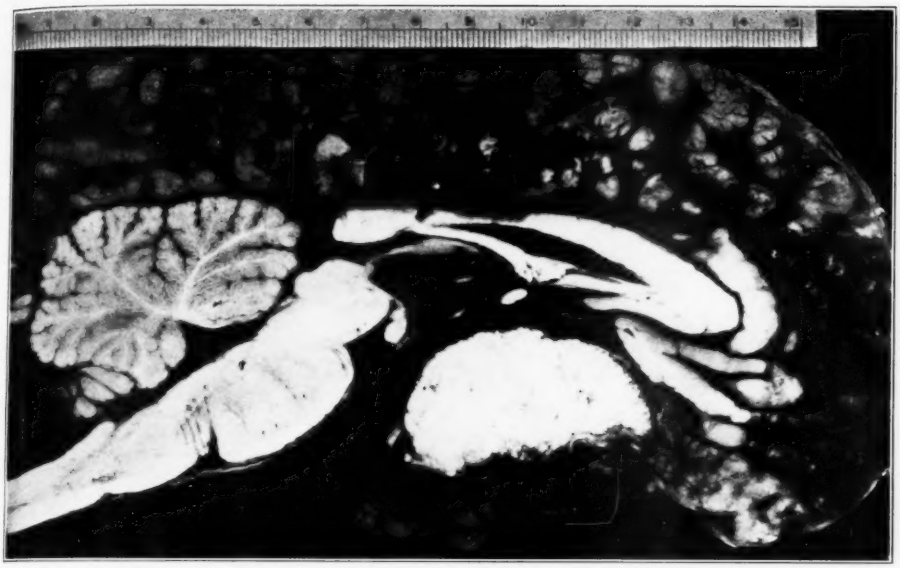
DESCRIPTION OF PLATE

PLATE 179

FIG. 1. Sagittal section of the brain. The large meningioma lies beneath and anterior to the third ventricle. The hypothalamus, optic chiasm and neighboring structures which have been dislocated dorsally are seen resting on the upper surface of the tumor.

FIG. 2. Sagittal section of the body of the sphenoid bone. The greatly thickened tuberculum sellae is seen just anterior to the sella turcica. It is obviously composed of spongy bone. Its entire thickness through to the dorsal wall of the sphenoidal sinus has been involved by tumor. Natural size.

FIG. 3. Photomicrograph of the bone of the tuberculum sellae. Tumor tissue typical of a meningioma is seen filling the spaces between the bony trabeculae. The tumor is composed of the usual spindle-shaped cells arranged in bundles and whorls. Hematoxylin and eosin stain. × 125.



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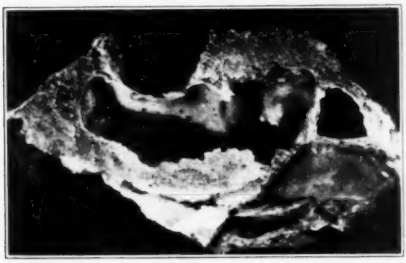

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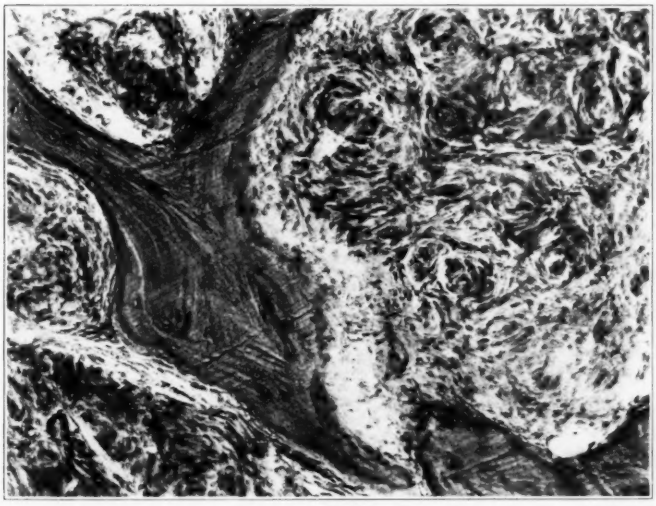

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Bucy and Kredel Meningioma of Tuberculum Sellae

PRIMARY INTRAMEDULLARY NEUROGENIC SARCOMA OF THE ULNA *

REPORT OF A CASE

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INTRODUCTION

The purpose of this paper is to record the occurrence of a tumor, primary in the ulna, having the characteristic structure of a perineurial fibroblastoma and presenting some histological evidence of malignancy.

The problems of histogenesis and classification of the tumors of nerves are still under dispute. This is especially true of the relation of the solitary tumors found on nerves to the generalized neurofibromatosis of von Recklinghausen. Penfield [1] in a recent publication has given an illuminating discussion of the question. To him the multiple fibrous nodules of von Recklinghausen's disease — the true neurofibromas — seem perhaps not neoplastic but rather of the nature of a reactionary fibrosis to some irritation, possibly neurotrophic, dependent upon congenitally faulty nerve insulation. Such nodules are tangled masses of collagenous tissue lacking any definite arrangement, and in their depths there are often to be found apparently intact nerve fibers.

Quite distinct from these knots of fibrous tissue is the truly neoplastic and usually solitary tumor mass with definite organoid structure found in connection with nerves. This type of tumor was originally called a neurinoma, or less exactly a neurofibroma. Subsequent study has given rise to two divergent concepts of its histogenesis. One group, chiefly the French investigators, believe the tumor to be derived from the cells of the sheath of Schwann, hence neurectodermal in origin, and call it the Schwannoma or peripheral glioma. The other group, whose view Penfield supports, claims the type cell to be the endoneurial fibroblast, and designates the tumor as a perineurial fibroblastoma. The most familiar example is perhaps

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the variously named intracranial tumor of the acoustic nerve, but localized tumors of the same type are commonly seen in the most varied places throughout the body. They are almost always slowly growing and well encapsulated tumors, though malignant change sometimes supervenes, especially in deep seated ones or following incomplete surgical removal.

Such growths are composed of parallel intertwining bundles of fairly mature fibroblasts, which show invariably a tendency to palisade arrangement of nuclei and often well marked undulant whorls. Between the cells is a moderately abundant quantity of fibrillary intercellular substance, very fine in texture and coloring only slightly with stains for collagen. Appropriate silver impregnation methods show that a considerable proportion of this intercellular substance is reticulum, in the form of long, straight, coarse fibrils, similar to the endoneurial reticulum described by Laidlaw.[2] Such reticulum stroma is a very constant feature, and together with the peculiar cell ordination is pathognomonic of the perineurial fibroblastoma. Fibroglia fibrils are often, but not always, demonstrable on the cells. Fat laden macrophages are frequently present, sometimes in great numbers. Areas of myxomatous degeneration with widely spaced stellate cells are often seen in the larger tumors.

Unlike the masses of von Recklinghausen's disease these tumors but rarely contain nerve fibers and then only at the periphery. Indeed their sole relation to generalized neurofibromatosis appears to be that occasionally within one of the multiple nodules a perineurial fibroblastoma may arise as a new and independent growth by a process of neoplastic release of included endoneurial fibroblasts.

Report of Case

The patient from whom the tumor to be described was obtained was a white painter, 55 years of age. He entered the hospital complaining of a swelling of the right forearm, which had been gradually increasing in size since first noticed a year previously. The swelling seemed to have caused no disturbance of motion or sensation, and only a mild aching pain in damp weather. About 6 months previous to noticing the tumor he had fallen, injuring his right arm, but not severely enough to prevent his working.

On examination he presented a large fusiform swelling 12 cm. in

greatest diameter in the middle third of the right forearm. The mass was hard, fixed to the ulna and not tender. The overlying skin was not warm or reddened. In spite of the size of the tumor, pronation and supination were free and complete. The record makes no mention of the presence of any of the stigmata of generalized neurofibromatosis.

X-ray plates (Fig. 1) showed the middle third of the ulna to be occupied by an expanding fusiform tumor arising within the medullary cavity and pushing a fairly complete thin shell of cortical bone before it. Within the mass, thin bony septa divided the tissue into large loculi, giving a marked "soap-bubble" appearance. No periosteal reaction triangle was present and there was no new bone formation in the tissue. X-ray diagnosis lay between benign giant cell tumor and some atypical form of fibrosarcoma.

Surgical exploration was decided upon. The ulna was exposed, the bony shell of the tumor broken through and the soft tumor tissue curetted out. The cavity left was cauterized with phenol and filled as well as possible with soft parts.

The tumor tissue removed was sent to the laboratory for frozen section diagnosis during the operation. A considerable quantity of rather soft, pinkish gray, friable tissue with small yellowish spots, larger translucent myxomatous areas and a few bony spicules was received. Frozen section showed a tumor consisting of loosely arranged spindle and stellate cells with very little delicate collagen between them. On the basis of rare mitoses and more frequent tumor giant cells a diagnosis of a slowly growing sarcoma was made, and because of the whorls, seen imperfectly in the unfixed section, it was suggested that the tumor might be of neurogenic origin.

Three days following operation celloidin sections were available. As in the frozen section, occasional mitoses and fairly numerous tumor giant cells were present. The whorls and palisades were now quite plainly evident and, accordingly, a diagnosis of a slowly growing, well differentiated neurogenic sarcoma was made.

Because of the highly malignant character of the cases of neurogenic sarcoma reported by Geschickter,[3] and others, surgical consultants were almost unanimous in advising amputation. After X-ray plates of the chest and pelvis had shown no evidence of metastases, the arm was amputated 6 cm. above the elbow. The large nerves at the site of amputation were found grossly normal in

appearance. The patient made an uneventful recovery and was discharged 2 weeks after operation. At the time of writing, 20 months after operation, he has shown no signs of recurrence or metastasis.

The ulna obtained from the amputated arm showed on the medial aspect a deep, crater-like defect 10 by 4 cm., filled with the flexor muscles. Between muscle and bone was some hemorrhage, and next the bone a narrow incomplete zone of grayish tissue. In microscopic section this appeared to consist only of granulation tissue and a small amount of newly proliferating bone. No recognizable tumor tissue was present. The preservation of free rotatory movement, in spite of the size of the tumor, was apparently due to the fact that the tumor expanded the cortex of the ulna only on the medial side and hence did not interfere with the motion of the radius.

Material and Technique

The following description is based on the material removed by curettage at the first operation. The tissue was fixed in Zenker's fluid and in 10 per cent formalin. Paraffin sections were stained with phloxine-methylene blue, phosphotungstic acid hematoxylin, hematoxylin and eosin, Weigert's elastic tissue stain, Mallory's aniline blue connective tissue stain and Masson's trichrome modification of it, and del Rio-Hortega's silver carbonate for reticulum, as modified by Foot for Zenker-fixed paraffin sections. Frozen sections of formalin-fixed tissue were stained with scharlach R and by the Smith-Dietrich method for lipoids. The blocks from which they were cut were mordanted, embedded in celloidin, and sections stained by the classical method of Weigert for myelin. Frozen sections were also impregnated by the Gros-Schultze and Bielschowsky methods for nerve fibers, and by a method devised by Penfield [4] for oligodendroglia and Schwann cells.

Description of Tumor

Microscopically the tumor consists of a loose but cellular tissue made up of spindle and stellate cells. In the greater portion they are arranged in the form of random bundles, but scattered here and there are numerous, loose, concentric whorls of large size, some in the form of longer undulant structures in which there is a tendency to palisading of nuclei (Fig. 2). The cells are nowhere closely packed

but are separated by a moderate amount of very delicate, fibrillary intercellular substance. Occasional areas are edematous, and both cells and fibrils are few and widely separated but preserve the suggestion of whorls (Fig. 3). No coagulum is present in the spaces between them. There are also numerous large areas in which the tissue is closely packed with great numbers of phagocytes having a rather uniform, foamy cytoplasm (Fig. 4). The vessels are sparse and are for the most part small, well formed arterioles and capillaries. In a few sections there are coarse strands of dense, collagenous connective tissue which probably represent parts of the fibrous periosteal capsule.

On closer examination the cells are seen to be of fairly large size and spindle, stellate or sometimes round in shape. The cytoplasm is moderately abundant, dense, homogeneous and basophilic, and in the round cells sometimes granular or vacuolated. The nuclei are vesicular with a medium amount of evenly distributed chromatin and one, occasionally two or more, large nucleoli. Multinucleated forms are exceedingly numerous, constituting the majority of cells in many fields. Here and there they have the shape of multinucleated ribbons of cytoplasm, somewhat resembling the proliferating neurilemma in the regenerating end of a cut nerve (Fig. 5). There is, however, no suggestion of a collagenous sheath about them. Some cells appear to have processes surrounding and forming a partial sheath for masses of material resembling segments of myelin sheaths. Many others contain round or oval masses, or vacuoles of similar appearing material.

With phosphotungstic acid hematoxylin the delicate, intercellular fibrillary material is colored pale reddish brown, or is almost unstained. Fibroglia fibrils are well shown in the coarse, adult connective tissue fragments of the capsule, and in the adventitia of the larger vessels. The tumor cells have rarely one or more long, delicate fibrils coursing over the cell body (Fig. 6). These fibrils are found on the spindle cells and rarely on the stellate forms, but not on the swollen, multinucleated giant cells. More commonly, perhaps, the tails of the spindle cells stain heavily in the manner of spongioblasts. Occasionally a small group of centrosomes is visible near the nuclei in the large multinucleated polygonal cells.

Turning from the cells to a consideration of the intercellular substances, as displayed by suitable technique, the aniline blue con-

nective tissue stain reveals a large amount of finely divided collagen, and the more precise and powerful trichrome modification of Masson shows even more, between the cells and crosswise in the whorls. The abundance and character of this material is demonstrated fully, however, only by a silver impregnation such as the Hortega-Foot method. Sections so prepared show a great quantity of long, straight or wavy, wire-like fibrils running between the cell bundles and circularly and crosswise in the whorls (Fig. 7). A large amount of the intercellular material in this tumor is silver-positive, in other words it is reticulum. Only the coarser masses, some doubtless remains of preëxisting tissue, stain as collagen in the metallic impregnation.

The tumor cells apparently produce no elastic tissue. Rarely short fragments of elastic fibrils may be found among the tumor cells, and the few dense masses of adult connective tissue already mentioned contain fairly numerous, large elastic fibers. All these, however, appear most likely to be derived from preëxisting tissue, possibly the periosteum.

The large accumulations of fat-laden phagocytes are a curiously frequent, though not pathognomonic, feature of the perineurial fibroblastoma connected with both the acoustic and peripheral nerves. They are a very prominent item in this tumor and no entirely satisfactory explanation can be given for their presence. Where few in number they usually cluster about the adventitia of small vessels. In other places they fill whole low power microscopic fields, yet no evidence of degeneration is visible in the adjacent tumor tissue. They are far too numerous to have resulted from the destruction of included myelinated nerve fibers, as Wlassics [5] suggested. It is possible that the lipoid material is derived from the fat of the marrow replaced by the tumor. Against this origin, however, is the fact that fatty macrophages, while commonly seen in inflammatory lesions, are but rarely present in any of the varieties of bone tumors.

If one accept the Schwannian origin of the perineurial tumors, the attractive explanation suggests itself that the tumor cells, like normal Schwann cells, are producing myelin and discharging it free into the tissue where it is picked up by phagocytic cells. The phagocytes stain brilliantly with scharlach R, and occasional tumor cells, both in their neighborhood and at a distance, contain droplets staining as fat. However, the Smith-Dietrich reaction for lipoids is negative, save for a few granules in rare tumor cells, and the Weigert

method for myelin reveals no trace of myelinated nerve fibers or free myelin in the tissue. In unstained frozen sections examined by polarized light much of the fatty material in the phagocytes is doubly refractile.

No necrosis is seen in the tumor. The vessels are few in number, small and well formed, and do not show the hyaline degeneration often described in these tumors. Many of the vessels have a moderate perivascular lymphocytic infiltration. A few small hemorrhages, probably the result of trauma incident to curettage, are scattered in the tissue.

Discussion

This tumor is classified with the perineurial fibroblastomas of the acoustic and peripheral nerves because, like them, it presents a combination of spindle cell and myxomatous tissue in which the cells are often arranged in whorls and palisades, and have between them a delicate fibrillary stroma consisting in large part of reticulum. Probably the strongest evidence of relationship is the presence of striking numbers of whorls and palisades differing only in looseness of texture from those of the generally recognised perineurial fibroblastomas.

The reticulum stroma is also a very characteristic feature, as Penfield has pointed out, and is uniformly present in all of our specimens of perineurial tumors. The work of Mallory and Parker [6] has demonstrated that reticulum is the same chemical substance as collagen in merely a finer state of subdivision, yet it is certainly significant that the stroma of all perineurial tumors should be composed quite largely of this finely divided form of collagen. Reticulum in small amount is present in the stroma of most tumors, but according to the investigation of Foot and Day,[7] it forms the predominant stroma only in rapidly growing tumors. The perineurial fibroblastomas, however, including the case here reported, are slowly growing, so that the constant presence of large quantities of reticulum is a diagnostic feature of considerable importance and suggests an inherited manner of cellular growth maintained even in neoplasms.

The intramedullary position of this tumor is well shown in the accompanying X-ray plate (Fig. 1). This intramedullary position of a perineurial fibroblastoma is, as far as I can ascertain, unique. The nearest resemblance seems to be a case reported by Brooks and Lehman,[8] in which a periosteal cyst of the tibia formed one of multi-

ple bony deformities associated with generalized neurofibromatosis. The tissue removed is described simply as "typical neurofibroma as in the skin," but no details or illustrations are given.

A considerable number of tumors designated as neurogenic sarcoma are reported in the literature, but all seem to have been primary in the soft parts and to have involved bone only secondarily. Judging from the published illustrations they were all rapidly growing and highly malignant tumors, so anaplastic that the neurogenic origin of many is by no means clear.

The tumor in our case no doubt took origin from one of the sparse nerve trunks within the marrow cavity of the ulna. Nerve fibers in bone, usually accompanying vessels, are described in most textbooks of histology, and rarely in aplastic marrows small groups of myelinated fibers may be seen.

The classification of this tumor as a slowly growing sarcoma depends, perhaps rather empirically, as with other mesodermal tumors, upon the preponderance of cells over intercellular substance and upon the occurrence of occasional mitotic figures. The malignancy is certainly of a low grade and with such complete surgical removal recurrence or metastasis seems unlikely to occur.

A discussion of the vexed problem of Schwann cell versus fibroblastic origin of this tumor has been purposely avoided from a belief that to be of value such discussion would require a wider base than that afforded by a single case. Because of the opportunity offered by the many clearly visible tumor cells a trial was made of a modification of Hortega's method for oligodendroglia devised by Penfield,[4] and said to stain also the Schwann cell selectively. No appearance of specific staining of the tumor cells could be produced.

Summary and Conclusions

A case of a solitary intramedullary tumor of the ulna presenting the histological structure of a perineurial fibroblastoma and some evidence of sarcomatous growth has been described in detail.

The occurrence of tumors of the perineurial type primarily in bone must be exceedingly rare, this apparently being the first case recorded. Estimate of their biological character is accordingly uncertain, but on histological evidence they seem, unlike the neurogenic sarcoma of soft tissues, to be a tumor of low grade malignancy.

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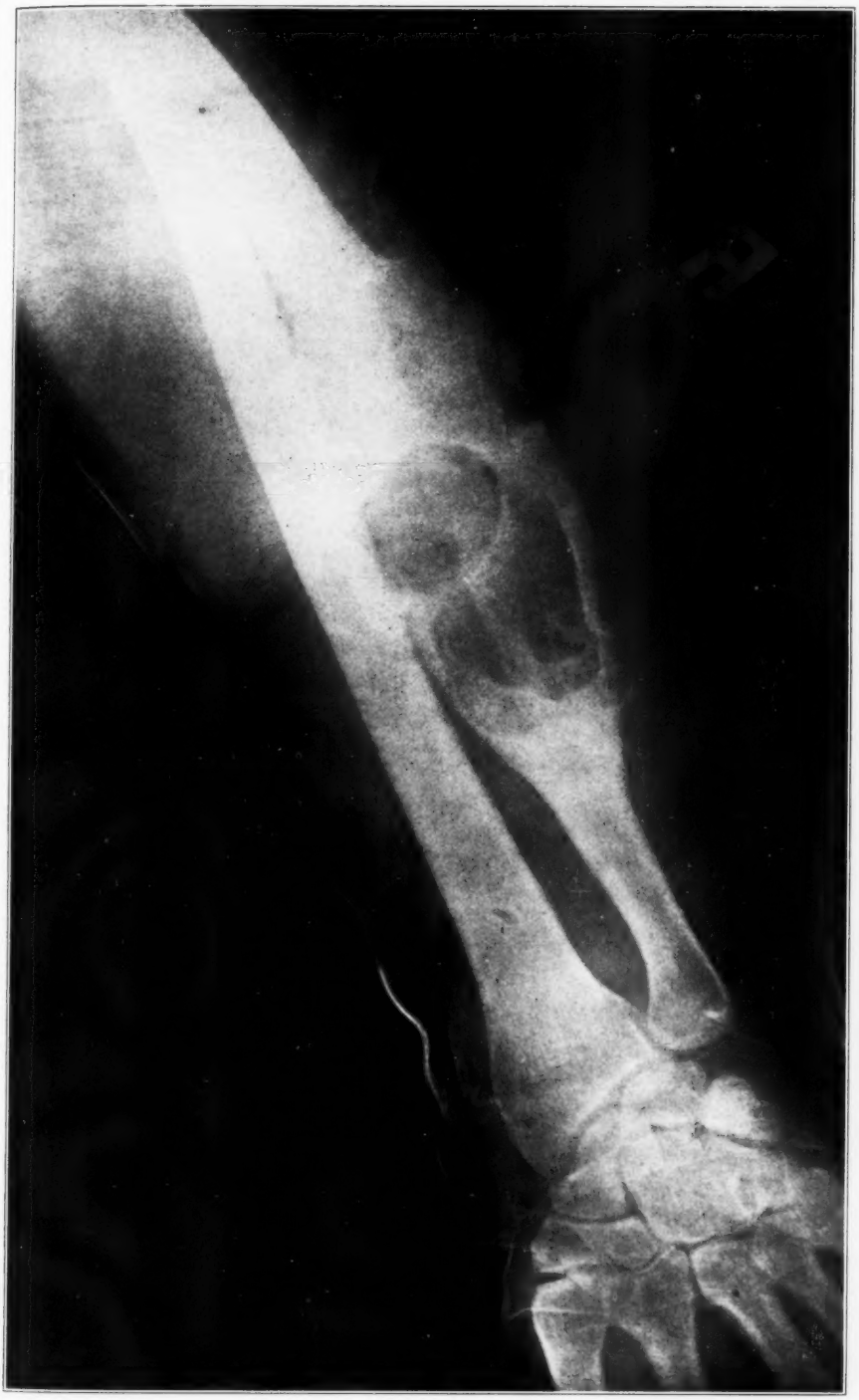
DESCRIPTION OF PLATES

PLATE 180

FIG. 1. Anteroposterior plate of the right forearm showing the tumor in the ulna.



Peers Primary Intramedullary Neurogenic Sarcoma of Ulna

PLATE 181

FIG. 2. Cellular portion of the tumor, showing whorls and a loose palisade.

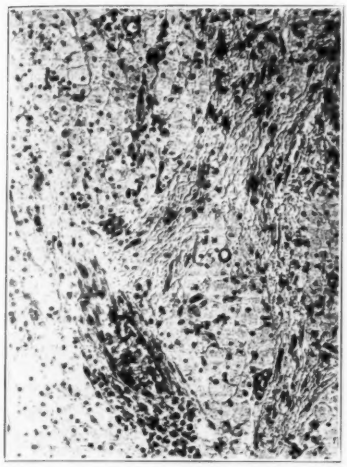
FIG. 3. Area of myxomatous type of tissue.

FIG. 4. Field showing extensive accumulation of fat-laden phagocytes. Lymphocytic infiltration about a small arteriole.

FIG. 5. Multinucleated tumor giant cell having the form of a ribbon of cytoplasm.

FIG. 6. A fibroglia fibril on the surface of a tumor cell. Phosphotungstic acid hematoxylin stain.

FIG. 7. Hortega-Foot impregnation showing the abundance and complex arrangement of reticulum in a whorl.



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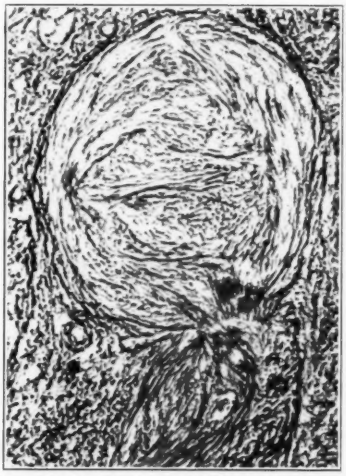

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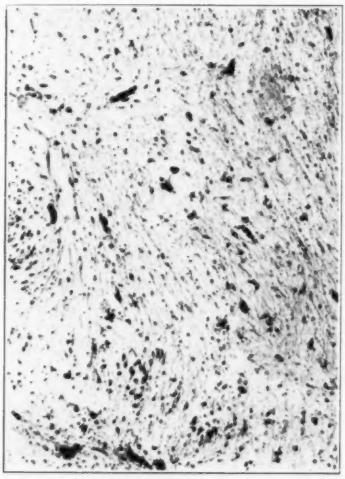

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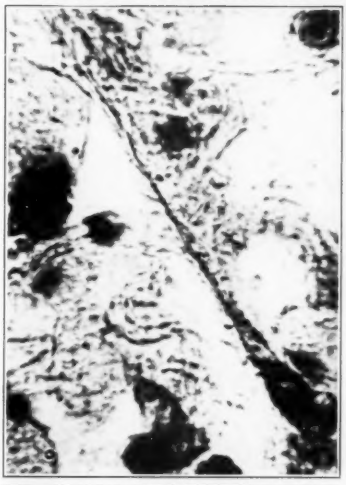

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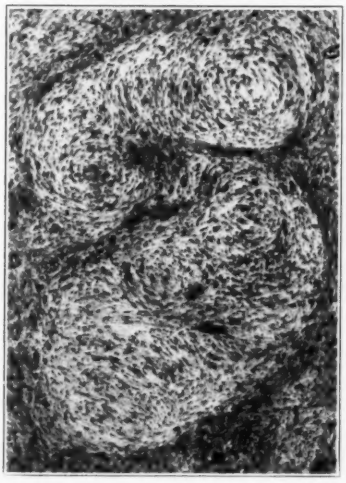

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5

Peers

Primary Intramedullary Neurogenic Sarcoma of Ulna

THE RELATION OF INCREASED INTRA-ABDOMINAL PRESSURE TO THE LIVER LESIONS OF ECLAMPSIA *

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Attention has been directed since 1886 to the occurrence of hepatic lesions in eclampsia. In that year Jürgens [1] and in 1888 Klebs [2] noted the occurrence of hemorrhagic hepatitis. In 1890 Pilliet [3] described the microscopic appearance of the liver lesions in 12 cases of eclampsia. Schmorl,[4] in a monograph based upon the autopsies of 17 women dead of eclampsia, stated that he had found in every case lesions of the liver which he believed were so characteristic that their presence justified the diagnosis of eclampsia without further knowledge of the history of the case. Microscopically these lesions consisted of areas of necrosis, with or without hemorrhage, located in the neighborhood of the smaller portal vessels, that is, a peripheral necrosis rather than the central necrosis commonly found in a variety of conditions. Many investigators confirmed these observations within the next few years: for example Bouffe de Saint Blaise [5] demonstrated such lesions in the livers of 42 consecutive cases and in 1902 Schmorl [6] reported finding them in 71 of 73 autopsied cases of eclampsia, the remaining 2 cases showing fresh complete thrombosis of the portal vein. Williams [7] recently stated that he had been able to demonstrate similar lesions in all the eclamptic livers that he had examined and agreed with Schmorl that such lesions occurred in no other condition. So characteristic are these lesions that many observers believe they represent the primary lesion of eclampsia, the manifestations of which are considered due to an impairment of hepatic function, "hepatotoxemia." There are other physicians,[8, 9] however, who report that the livers of eclamptic patients may occasionally show very little abnormality. Theobald [10] states that typical eclamptic liver lesions may be found rarely in patients dying of

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general peritonitis and in those with pneumonia. Mallory,[11] many years ago, suggested that the appearance of hemorrhages in the liver in eclampsia could probably best be explained as the result of the purely mechanical forces involved in the convulsions. He noted the occurrence of similar lesions in the liver of a man dying from meningitis. Quite recently Theobald [10] has revived the mechanical theory for the etiology of eclamptic liver lesions. He has performed a number of interesting experiments on animals in which he believes that he has produced the eclamptic type of liver lesion by repeatedly raising the intra-abdominal pressure. If it can be shown that liver injury is a secondary phenomenon, the theory that eclampsia is a primary hepatotoxemia must be abandoned. Theobald's observations are, therefore, of such importance that it was considered advisable to repeat them. The technique used by Theobald [10] in his experiments was as follows.

"The dog was given an injection of morphine sulphate gr. i and half an hour later anaesthesia was induced with a mixture of equal parts of chloroform and ether. The animal was then tied on its back and a litre (sometimes a little more) of sterile normal saline solution was introduced with all antiseptic precautions into the peritoneal cavity through a trocar inserted in the middle line. The reason for the introduction of the saline was because without it, it was found impossible to raise the intraperitoneal pressure much above 40 cm. of saline solution. The fluid having been run in, two dusters were placed round the abdomen and drawn tight, by which manoeuvre the intraperitoneal pressure was raised to between 80 and 100 cm. of saline solution. This manoeuvre will, for the sake of convenience, be referred to as a 'fit,' each of which lasted two minutes unless the contrary is stated."

His first animal was sacrificed 3 hours after the experiment in which the intra-abdominal pressure had been raised 9 times. The liver is described as follows: "The cells are swollen and granular, the nuclei stain badly and the sinusoids are widely dilated. The feature of interest, however, is the fact that Glisson's capsule has been most severely affected and in nearly every area the cells are swollen and the structures, including the bile ducts, are seen to be disintegrated." These changes cannot be made out clearly in the halftone reproduction (Fig. 1 [10]) of the photomicrograph. A second animal received only morphine. The number of "fits" and the time of sacrificing

are not stated. No changes are apparent in the reproduction (Fig. 2 [10]) of the photomicrograph, although it is stated that changes similar to those quoted above are present. A third animal, receiving as an anesthetic the chloroform-ether mixture and having its intra-abdominal pressure raised 5 times one day and 6 times the next day, was sacrificed 24 hours later. The liver showed "extensive degeneration and necrosis, chiefly central but extending in not a few areas to the periphery of the lobules." These changes are illustrated in the photomicrograph (Fig. 3 [10]), and do not appear at all characteristic of the eclamptic type of lesion in woman. Another animal killed 14 days after having had its intra-abdominal pressure elevated 3 times in one day and 5 times on the next day, showed extensive necrosis, the location of which is not specified in the text and cannot be determined from the photomicrograph (Fig. 4 [10]). A pregnant cat, killed 18 hours after the intra-abdominal pressure had been raised, showed extensive degeneration in the liver "with no particular lobular distribution." A dog, whose intra-abdominal pressure had been raised 30 times at 3 minute intervals, each time for 30 seconds, was killed 24 hours later. "Sections (of the liver) show large areas of necrosis, chiefly central, and a few areas of hemorrhagic necrosis situated in the periphery of the lobules."

Experimental

Our procedure was identical with that of Theobald, except for the fact that morphine was not employed in any instance and ether alone was used as an anesthetic in 7 dogs. A mixture of equal parts of chloroform and ether, as employed by Theobald, was used in 4 other dogs. The protocols of the 11 experiments follow.

Dog No. 29: Ether anesthesia. Intra-abdominal pressure raised 18 times, immediately following which respirations ceased, 72 minutes after the first elevation of pressure. Autopsy revealed edema of the lungs and an elevated diaphragm. The liver was grossly and microscopically normal.

Dog No. 25: Ether anesthesia. Intra-abdominal pressure raised 10 times, immediately following which respirations ceased, 40 minutes after the first elevation of pressure. The liver was grossly and microscopically normal.

Dogs Nos. 23, 24 and 28: Ether anesthesia. Intra-abdominal pressure raised 20 times during a period of 80 minutes in each animal. Each dog was sacrificed 72 hours after the completion of the experiment. All three livers were grossly and microscopically normal.

Dog No. 26: Ether anesthesia. Intra-abdominal pressure raised 15 times during a period of 60 minutes on 1 day and this repeated 2 days later. The

animal was sacrificed 72 hours after the 2nd day on which the pressure was raised. The liver was grossly and microscopically normal.

Dog No. 27: Ether anesthesia. Intra-abdominal pressure raised 10 times within 40 minutes on 1 day, and 5 times within 20 minutes 5 days later. The animal was sacrificed 5 days after the 2nd day on which the pressure was raised. The liver was grossly and microscopically normal.

Dog No. 20: Anesthesia maintained with equal parts of chloroform and ether. Intra-abdominal pressure raised 10 times. The animal was sacrificed 72 hours later. The liver showed widespread central necrosis, occasionally extending to the periphery of the lobule.

Dog No. 30: Anesthesia maintained with equal parts of chloroform and ether. Intra-abdominal pressure raised 10 times within a period of 40 minutes on 1 day, and this repeated 72 hours later. The animal was sacrificed 72 hours after the 2nd day on which the pressure was raised. The liver showed extensive central necrosis, occasionally extending to the periphery of the lobule.

The next two animals (Nos. 21 and 22) were merely anesthetized with equal parts of chloroform and ether.

Dog No. 21: The anesthesia was maintained for 1½ hours. This animal received more of the anesthetic mixture than either of the above dogs, Nos. 20 and 30. It was sacrificed 72 hours later. The liver was grossly and microscopically normal.

Dog No. 22: The anesthesia was maintained for 1½ hours. This dog received much less of the anesthetic mixture than dogs Nos. 20, 30 and 21. It was sacrificed 72 hours later. The liver showed most extensive central necrosis with only a border of cells remaining intact about the periphery of the lobules.

Discussion

The above observations on dogs Nos. 20 and 30 confirm those of Theobald, inasmuch as extensive necrosis of the liver, chiefly central, but occasionally extending to the periphery of the lobule, occurred when the intra-abdominal pressure was raised in animals which had received chloroform-ether anesthesia. However, 1 of 2 animals receiving merely this same anesthetic, developed even more extensive liver lesions. It is likewise to be emphasized that 7 animals anesthetized with ether alone, 5 of which survived 3 or more days, although subjected in most instances to even more numerous elevations of the intra-abdominal pressure than Theobald's animals, failed to show any lesions of the liver. The significance of the use of chloroform as an anesthetic is apparent. It is so well known that chloroform can produce extensive central necrosis of the liver that this need not be enlarged upon here. It is also apparent that the lesions produced by Theobald [10] are entirely consistent with chloroform necrosis and are not at all characteristic of the periportal lesion of eclampsia.

Summary and Conclusions

1. Elevation of the intra-abdominal pressure, according to the technique of Theobald, was without effect upon the livers of 7 dogs anesthetized with ether.

2. A chloroform-ether anesthetic mixture, such as that employed by Theobald, resulted in extensive central necrosis of the liver in 3 of 4 dogs, only 2 of which were subjected to increases of intra-abdominal pressure.

3. The theory that the liver lesions of eclampsia are due to increased intra-abdominal pressure, although an attractive hypothesis, must be considered as yet unproved.

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CHANGES PRODUCED IN THE CENTRAL NERVOUS SYSTEM OF THE MOUSE BY THE VIRUS OF ST. LOUIS ENCEPHALITIS *

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Transmission of the virus of St. Louis encephalitis to mice has been reported by two groups of workers: (1) Muckenfuss, Armstrong and McCordock,[1] and (2) Webster and Fite.[2] The latter workers used a special strain of mice peculiarly susceptible to neurotropic virus, and they also found that Swiss mice would contract the disease, but that the stock strain of mice used in the Rockefeller Institute was affected in a "relatively small percentage of animals." Muckenfuss and associates demonstrated that stock strains of mice available in St. Louis were uniformly susceptible. Both of the above groups of authors have briefly described the symptoms and lesions produced in mice by the virus.

MATERIAL

Four strains of virus were used: three strains isolated in this laboratory, namely, the Freeman, Barnes, and Daily strains, and one obtained by Webster designated as No. 3. Two hundredths of a cubic centimeter of 10 per cent brain emulsion was inoculated into the brains of white mice of the Swiss strain and white mice obtained from three breeders in the St. Louis area.

Several hundred brains were removed with the least possible traumatization and fixed in Zenker-acetic solution. Paraffin sections of blocks taken from various levels were stained with hematoxylin and eosin. For particular studies animals were killed at definite intervals after inoculation, or when moribund. Special fixatives and staining methods were used to demonstrate particular changes.

COURSE OF ILLNESS

The mice usually remain well until the 5th day after inoculation, but occasionally they show signs of illness and die on the 4th day, when highly virulent material has been used. The first signs are a ruffing of the fur and a hunched posture. At this stage an exaggerated response is obtained on stimulation. Then the mouse may assume the "encephalitic position,"[3] sitting erect with hind legs

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spread apart and the tail used as a support. Tremors of extremities, head and tail are usually observed. Convulsions and paralyses, both spastic and flaccid, are common and occasionally the mice run in circles. Some animals apparently have disturbance of vision for they run into objects and fall off the edge of the table.

The disease may progress very rapidly and kill the mouse in a few hours or it may be protracted for several days. In the latter case the paralyses frequently become generalized and the respirations slow and shallow.

Pathology

Webster and Fite [2] have described the lesions produced in mice by the St. Louis encephalitis virus as: "accumulations of mononuclear cells in the pia, about the blood vessels in the brain and spinal cord and in scattered foci. The pyramidal cells of the cornu ammonis and lobus pyriformis are injured, thus disturbing the regular architecture of these regions. Certain nerve cells of the basal ganglia and anterior horn cells of the spinal cord appear damaged and collared by mononuclear cells."

Little need be said about the gross appearance of the brain. The surface vessels are usually injected and the brain frequently has a diffuse pinkish appearance. The site of inoculation in the left area striatum [4] at the left of the thalamus is generally apparent as a small red depression.

Microscopically the most striking and constant lesion is the collection of cells about vessels. This is observed in all parts of the brain and in the spinal cord. There are three types of cellular reaction, all of which may be exhibited in the same brain.

The first type consists of a collection of mononuclear cells in the Virchow-Robin space. The predominant cell in the perivascular cuff is the lymphocyte, but not infrequently there are also mononuclear wandering cells, some with elongated oval nuclei and scanty cytoplasm, others with kidney-shaped nuclei and abundant cytoplasm. The width of the cuff varies from one layer of cells to four or five. There is no cellular infiltration in the brain substance immediately surrounding the vessel (Fig. 1).

The second type of reaction is characterized by the presence of cells in the brain substance adjacent to vessels, but with none of the previously described cells in the Virchow-Robin space. With hema-

toxylin and eosin stain the cells consist of irregularly shaped nuclei and little or no cytoplasm. Some of the nuclei are elongated and rod-like with slightly blunted ends, others are club-shaped, and some are curved and twisted on themselves (Fig. 2). In material fixed in formol-ammonium bromide and stained by del Rio Hortega's method, many of these cells show the typical appearance of microglia (Fig. 4).

The third type is a combination of the first two. Here there is a perivascular cuff and also a proliferation of microglia in the brain substance about the vessel (Fig. 3).

All three reactions are usually present in the same mouse. Probably the most frequent type of involvement is that in which there is both a perivascular cuff and a collection of glia cells near the vessel. Cuffing alone is more common than the simple glia proliferation.

Mice vary individually, not only in the severity of reaction to the same dose of virus, but also to some extent in the type of cellular collections about vessels; thus in a particular lot some mice will show cuffing predominantly with only a slight glial reaction, while others display a glial response equal to or greater than the perivascular.

Different strains of white mice apparently show a slight variation in response to the virus. Swiss mice in general have more clearly defined and thicker perivascular cuffs. Mice obtained from three different stocks in the St. Louis area, among which there was probably more or less cross-breeding, seem, in general, to show smaller and less clearly defined cuffs with a somewhat greater proliferation of glia.

The reaction about vessels appears as early as the second day after inoculation. At this time it is not intense, although cuffs one to two cells thick and some microglia proliferation in the surrounding brain tissue are observed in the Swiss mice. The region of inoculation is first affected. By the 3rd day the lesions are scattered diffusely throughout the brain with less involvement of the cerebellum than of the cerebrum.

Degeneration of ganglion cells is another constant lesion. Various changes are observed, such as swelling, chromatolysis, and pyknosis of the nucleus, disappearance of Nissl granules with a homogeneously purple-staining of the cytoplasm, and finally such complete destruction of the cell that it stains a deep pink. The 2nd day after inoculation there is some degeneration of ganglion cells in an area

limited almost entirely to the region of inoculation. By the 3rd day the degeneration is apparent throughout the brain in a patchy distribution. Small areas of ganglion cells in Ammon's horn show the change frequently (Fig. 5). The patches of degeneration are often observed in the vicinity of a vessel. Most of the ganglion cells within the circumference of the glial reaction show changes, but the process rapidly diminishes beyond it.

In mice killed when moribund the widespread severe destruction of nerve cells is striking. No area is immune, but there are still islands of normal cells. The cortical substance in the occipital and striate areas, and in the parietal region overlying Ammon's horn, is more severely affected than are the basal nuclei and the brain stem. Neuronophagia is observed. Some of the Purkinje cells of the cerebellum take a deep purple stain with hematoxylin and eosin but rarely show the complete necrosis seen in cells in the cerebrum. The degeneration is more marked in mice inoculated with a heavy dose of the virus.

A third constant feature of the disease is an infiltration of the meninges with cells (Fig. 6). These cells are sometimes grouped about vessels but are usually scattered in the subarachnoid space. Lymphocytes predominate but there are also monocytes and an occasional polymorphonuclear leukocyte and plasma cell. The meningeal reaction varies in intensity and may be fairly marked as early as 48 hours after inoculation.

Focal collections of cells occur in brain tissue of mice less frequently than in human or monkey material. Occasionally, small collections of glia cells or of mononuclears apparently not related to a vessel are seen (Fig. 7). In order to determine more definitely the relation between these collections and vessels, serial sections of two mouse brains were made. All of the focal collections in these two brains appear to be a part of a cuff or a glial reaction about a vessel. The blood vessels and small capillaries are all congested, frequently to a marked degree. Hemorrhages into the perivascular space or into the brain substance are occasionally seen. No evidence of demyelinization is present in sections stained by Loyez' method. No intranuclear inclusion bodies are found in Giemsa preparations, nor are the cytoplasmic inclusions described by Nicolau and coworkers [5, 6] in normal mice obtained in Paris and England observed in the four strains of mice at our disposal.

The spinal cord shows well developed lesions. The three types of cellular response observed about vessels in the brain are frequently seen in the cord, but here the reaction is generally less severe. Degeneration of nerve cells, mainly the motor cells of the anterior horn, occurs (Fig. 8). Swelling of the motor cells appears as early as the 2nd day, before there is involvement about vessels. By the 5th day degeneration of the ganglion cells is marked. The cervical and upper thoracic regions of the cord are most frequently involved.

Sections of other organs were made in a number of instances. No notable changes are observed, except for the occasional presence of fat droplets in liver cells.

Discussion

Spontaneous encephalitis occurring in mice which appeared healthy during life has been described by Cowdry and Nicholson.[7] The protozoan-like parasites and the lesions in the brain were similar to those described in rabbits by several investigators.[8, 9, 10] Levaditi [11] has named the parasites "microsporidia," and further designated them as "*Encephalitozoön cuniculi*."

The lesions of spontaneous encephalitis in mice were described by Cowdry as follows: "Focal infiltrations are most frequently met with and perivascular, meningeal and subependymal infiltrations were seldom seen in their absence. It was . . . a common experience to find focal infiltrations when the other varieties were difficult to detect. . . . In many cases . . . it was possible to trace complete continuity between a meningeal infiltration on the surface, its extension into the brain substance as a perivascular infiltration about a penetrating blood vessel, and its termination as a focal lesion either at the end of a vessel or to one side of it. . . . The same kinds of cell were found to participate in the infiltrations quite irrespective of their location (meningeal, perivascular, focal, or subependymal). In all . . . specimens lymphocytes predominated, but a few plasma cells and macrophages were also noted. The latter were particularly numerous at the centers of the focal lesions in the presence of slight necrosis and in the absence of lymphocytes, which were clumped about the periphery."

We have not encountered evidence of spontaneous encephalitis in mice of the Swiss stock. Occasionally this condition was observed in mice of the St. Louis stocks. In one lot of a hundred mice, which

appeared in poor general condition, the brains of eight were sectioned as controls. Four had the lesions described by Cowdry and in three of these microsporidia were found. This is an unusually large proportion and in other apparently healthy lots from the same breeder evidence of spontaneous encephalitis was infrequent.

In general, the spontaneous type of encephalitis which we have encountered has corresponded with the description given by Cowdry. In one instance we found the parasites scattered among the cells infiltrating the meninges. In our mice we have sometimes seen glia cells in the focal reactions in addition to the mononuclear leukocytes and lymphocytes.

Cowdry described lesions in the cervical cord and stated that they probably extend farther down the cord. We have examined spinal cords from three mice in which the brain contained parasites and lesions. In each of these a mononuclear infiltration has been found in the spinal meninges as well as around vessels, and also collections of mononuclear and glia cells about a focus of necrosis. Ganglion cell degeneration has not been observed in these sections. Microsporidia were not demonstrated in the cord. The lesions were observed in the cervical, thoracic and lumbar regions.

Neither in the previous description of spontaneous encephalitis nor in our experience was a glial reaction seen about the vessels with cuffs, except when the cuffed vessel was near a focal reaction which contained glia cells.

The desirability of using a strain of mice free from epizoötic encephalitis when working with a neurotropic virus cannot be overemphasized. Even when the lesions are severe it is difficult to distinguish the spontaneous from the experimental type of encephalitis, but there are several criteria which help to differentiate the two. The presence of microsporidia is of course positive evidence of the epizoötic origin of the disease. Proliferation of microglia about the perivascular cuffs is rarely seen in spontaneous encephalitis, whereas it is a constant finding in the experimental type. Degeneration of ganglion cells of the brain and spinal cord is much less frequent and less severe in the former. On the other hand, the focal reactions are more numerous, more marked and of a slightly different character in the former. Of less importance is the tendency in the epizoötic disease for the infiltrations to extend from the meninges into the brain substance along vessels and in sheets. This is not so apparent

in experimental encephalitis, where cuffing is rather evenly distributed throughout the brain.

The pathological picture produced by the St. Louis encephalitis virus is comparable in humans, monkeys and mice. There is no significant difference in the lesions found in man and monkey. The process in mice seems more severe than in the other two species; in general the cuffing, ganglion cell degeneration and meningeal reaction are more marked. The glial reaction about vessels, which is common in mice, is rarely, if ever, seen in man and monkey, whereas the glial nodules which are common in the two latter are relatively infrequent in mice.

Summary

1. A description of the pathological lesions produced in the mouse by the St. Louis encephalitis virus is recorded.

2. A comparison is made between the lesions in the mouse and those in man and monkey.

3. A differentiation between the lesions in mice in experimental encephalitis and in epizoötic encephalitis associated with microsporidia is discussed.

Note: We wish to express our indebtedness to Dr. Howard A. McCordock for his helpful criticism and for his aid in preparing the photomicrographs.

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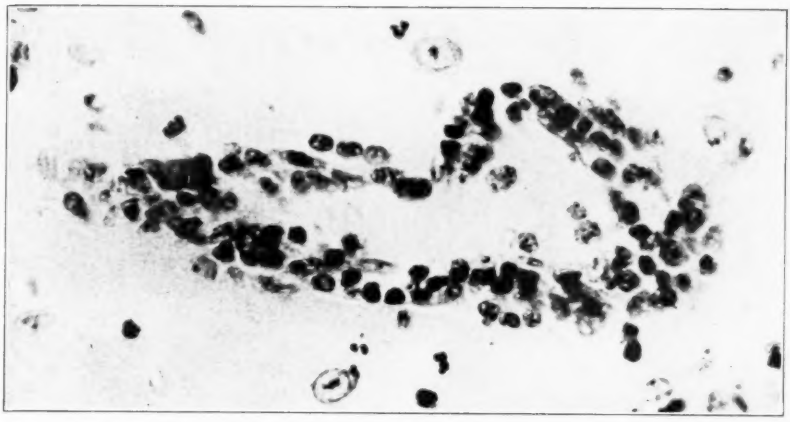
DESCRIPTION OF PLATES

Plate 182

Fig. 1. Vessel with collection of lymphocytes and mononuclear phagocytes in the perivascular space. The surrounding brain tissue is free from infiltration. Hematoxylin and eosin. × 700.

Fig. 2. Here the perivascular space is clear but in the brain tissue surrounding the vessel there is a collection of newly formed cells. At the left of the vessel there are several cells with curved and dumb-bell-shaped nuclei, which appear to be microglia. Several dark, shrunken nerve cells are apparent. Hematoxylin and eosin. × 700.

Fig. 3. A vessel with a perivascular collar of mononuclear cells. In addition, in the surrounding brain tissue there is a proliferation of microglia cells with irregularly shaped nuclei. Hematoxylin and eosin. × 700.



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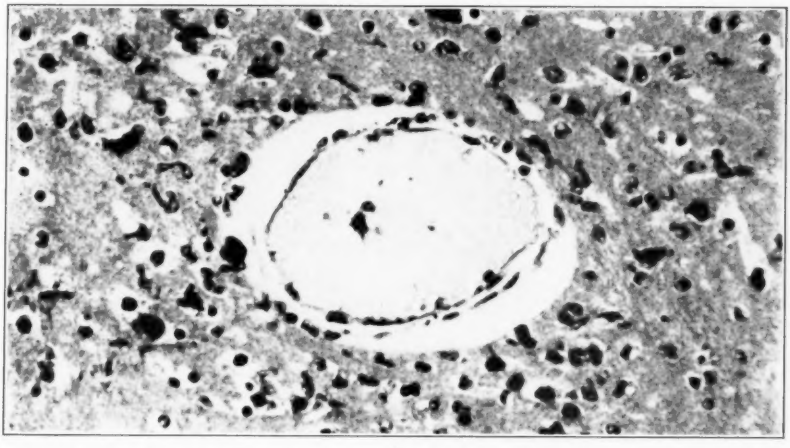

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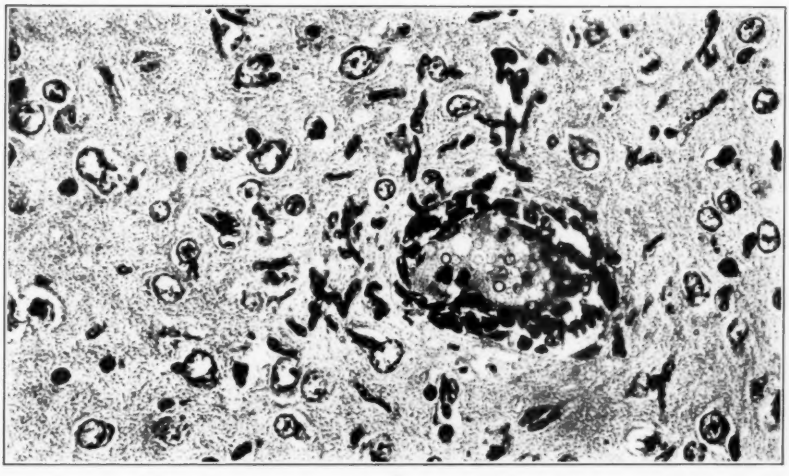

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PLATE 183

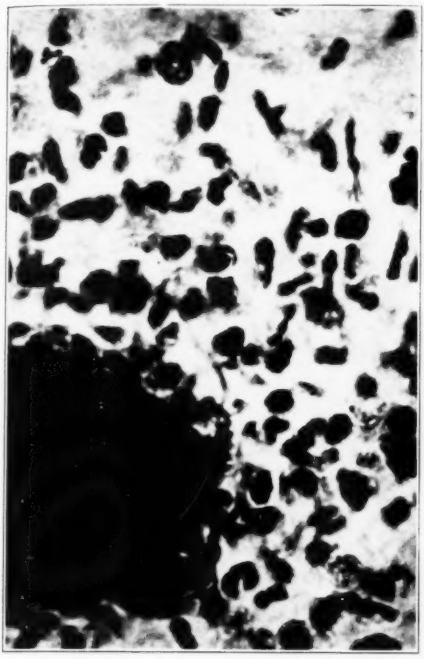
FIG. 4. A blood vessel is seen at the lower left, around which there is a collection of mononuclear and glia cells. Microglia are seen as irregularly shaped, elongated rods. In the upper right portion a microglia cell with processes is apparent. P. del Rio Hortega's silver carbonate method. × 700.

FIG. 5. Degeneration of ganglion cells in Ammon's horn. In a circumscribed area the degenerated ganglion cells appear dark and homogeneously stained. Adjoining this area normal ganglion cells are seen. Hematoxylin and eosin. × 300.

FIG. 6. Infiltration of meninges with lymphocytes and large mononuclear wandering cells. The infiltration extends down into the cortex around a vessel. Hematoxylin and eosin. × 300.

FIG. 7. Small focal collection of mononuclear and glia cells in region of basal nuclei. Hematoxylin and eosin. × 300.

FIG. 8. Degeneration of motor cells in anterior horn of spinal cord. Proliferation of glia cells is also present. Hematoxylin and eosin. × 700.



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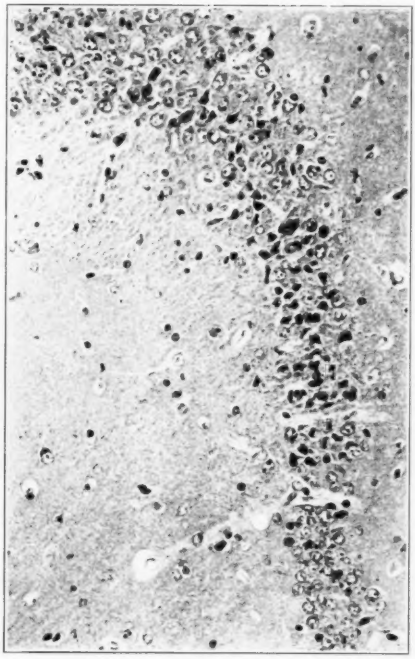

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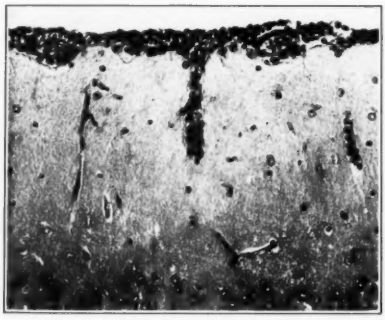

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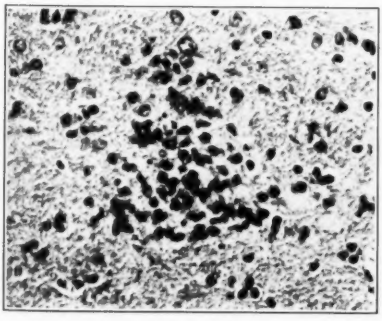

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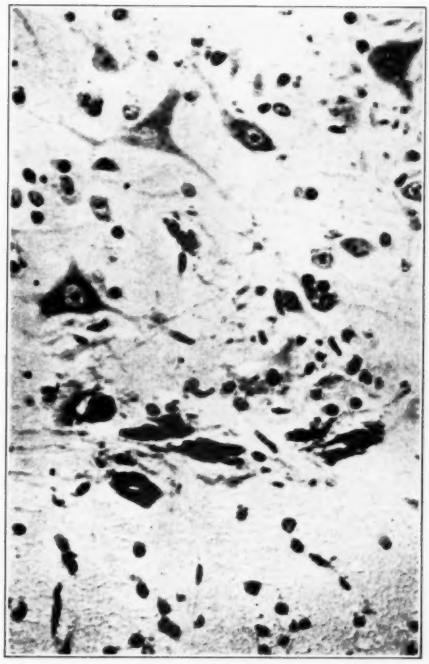

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Changes in Central Nervous System of Mouse

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